"UTILITY OF TOLUIDINE BLUE STAINING AND BRUSH BIOPSY IN PRECANCEROUS AND CANCEROUS ORAL LESIONS"

By

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Under the guidance of

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TO,

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List of abbreviations:

AJCC :American Joint Committee on Cancer

BB:Brush Biopsy

EBV:Epstein Bar Virus

HIV Human Immunodeficency Virus

HPV:Human Papilloma Virus

IHC : Immunohistochemistry

ISH :Insitu Hybridization

LOH:Loss of heterozygosity

MDSCC: Moderately Differentiated Squamous Cell Carcinoma

NCRP :National Cancer Registry Programme

OPD :Out Patient Procedure

PCR: Polymerase Chain Reaction

PDSCC: Poorly Differentiated Squamous Cell Carcinoma

PML : Potentially malignant Lesions

RFLP :Restriction Fragment Length Polymorphism

SCC: Squamous Cell Carcinoma

SEER :Surveillance Epidemiology and End Results

TB: Toulidine Blue

TNM :Tumor Node Metastasis

UDSCC :Undifferentiated Squamous Cell Carcinoma

UICC :International Union Against Cancer

WDSCC: Well Differentiated Squamous Cell Carcinoma

ABSTRACT

Objectives of study:

To evaluate the usefulness of toluidine blue and brush biopsy in the precancerous and cancerous oral lesions.

Study Design:

This is a prospective study of 172 patients with premalignant/malignant oral lesions who attended the outpatient clinics of Otorhinolaryngology and Dental Surgery at R. L. Jalappa Hospital and Research Centre, Kolar, were screened with in vivo toluidine blue staining oral brush biopsy and wedge biopsy. Statistical analysis was done using statistical software SPSS version 16. To test the diagnostic accuracy of the screening tests sensitivity, specificity and predictive value were calculated.

Results:

Out of 172 cases predominantly 127 cases were females and 45 were males. 84.3% had a habit of areca nut /tobacco chewing. Maximum number of cases were seen in buccal mucosa and tongue (75%). Toulidine Blue staining of oral lesions showed strong positivity in 72% of cases. Brush Biospy detected 69% cases as malignant. Combined evaluation of toulidine blue and Brush Biopsy showed sensitivity of 88% and 93% for premalignant lesions and malignant lesions respectively. The false negative cases were reduced by this combined technique.

Conclusion:

This study suggests that early detection of oral cancer is possible even at precancerous stage by using noninvasive, painless outpatient procedure of combined invivo

supravital staining of Toluidine blue and Brush biopsy. Combined evaluation of toluidine blue and brush biopsy has helped to increase the sensitivity and specificity in detecting premalignant lesions and also to minimize false negatives.

Key words : oral cancers; toulidine blue; brush biopsy

TABLE OF CONTENTS

List of Tables

List of Figures

Introduction

INTRODUCTION

Oral cancer is a global health problem with increasing incidence and mortality rates; around 300,000 patients are annually estimated to have oral cancer worldwide.[1]

In India, oral cancer represents a major health problem constituting up to 40% of all cancers and is the most prevalent cancer in males and the third most prevalent in females.[2]

Despite the easy accessibility of the oral cavity during physical examination, many malignancies are not diagnosed until late stages of disease. Multiple screening, detection techniques and different modalities of treatment to prevent progression of premalignant to malignant lesions have been tried. The early detection of cancer is of critical importance because survival rates markedly improve when the oral lesion is identified at an early stage and management becomes easier and less morbid.

Clinical diagnosis of squamous cell carcinomas of oral mucosa is not difficult when the lesion is obviously invasive or functional limitation is present. Conversely, it is more difficult to diagnose dysplasias and potentially malignant epithelial lesions. [3]

Supravital stain Toluidine blue has been used to mark the area for biopsy and to mark the full extent of premalignant lesion. It has been reported that toluidine blue stains premalignant and malignant lesions, but, not the benign lesions and normal mucosa.[2]

Toluidine blue is a member of thiazine group of metachromatic dyes.[4] In vivo staining may identify early lesions which could be missed on clinical examination[5]. Moreover it can outline the full extent of the dysplastic epithelium or carcinoma when excisions are planned[6]. It helps in selecting the biopsy sample site in premalignant lesions. Also, it can help the follow up of patients with oral cancers.[7] Histopathological diagnosis by scalpel biopsy is the gold standard for accurate diagnosis.[3,6] Patients often fear the routine biopsy as it is an invasive Out Patient (OPD) Procedure. Hence as an

alternative,brush biopsy which involves scraping of surface epithelium and is less traumatic,can be used as a routine Out Patient (OPD) Procedure procedure. In our centre we are trying to assess the efficacy and accuracy of TB in comparison of wedge biopsy.

This study will help us to develop expertise and experience in this procedure and determine the significance of an oral lesion prior to wedge biopsy and also help us to evaluate whether false negatives can be minimized.

AIMS AND OBJECTIVES

1.To evaluate the usefulness of toluidine blue and brush biopsy in precancerous and cancerous oral lesions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

History

The term *leukoplakia* was first used by Schwimmer in 1877 to describe a white lesion of the tongue, which probably represented a syphilitic glossitis.[8]

The vital staining method was used at first in medicine for detecting cervical dysplasia and carcinoma *in situ* in the 1960s.

Toluidine blue was first used by Richart in 1963 to stain uterine cervical carcinoma in situ.[1] Niebel and Chomet were the pioneers who used dye material to detect oral cancer in 1964.[9]

Oral brush biopsy was introduced to the dental profession in 1999, overcoming the limitations of traditional oral cytology.[10]

Earliest evidence of cancer is found among fossilized bone tumors, human mummies in ancient Egypt and ancient manuscripts. Oldest description of cancer was discovered in Egypt and dates back to approximately 1600 B.C.

The origin of the word *cancer* is credited to the Greek physician Hippocrates (460-370 B.C.), considered the "Father of Medicine." Hippocrates used the terms *carcinos* and *carcinoma* to describe non-ulcer forming and ulcer-forming tumors.

Anatomy of oral cavity:

The anatomy of oral cavity is shown in Figure 1 and Figure 2.

Oral cavity extends from vermilon border of the lip anteriorly to the junction of hard palate and soft palate posteriorly,inferiorly to cicumvallate papillae and laterally to anterior tonsillar pillars.

The subsites in oral cavity are upper and lower dentoalveolar ridges, anterior two third of tongue, retromolar trigone, floor of mouth, buccal mucosa including mucosa of lips and hard palate.

Lips: It is the transition from external skin to internal mucosal membrane that occurs at vermillon border.Orbicularis oris gives the sphincter like action.

Alveolar ridge: Lateral aspect is formed by mucosal sulcus by transition to buccal mucosa. In alveolar ridge ,medial margin is marked by transition to floor of mouth and on upper alveolar ridge,is the horizontal orientation to hard palate. The posterior margin of lower alveolar ridge is ascending portion of ramus of mandible, whereas it is the superior aspect of of pterygopalatine area for upper alveolus . The close proximation of mucosa to underlying bone facilitates early bone invasion for malignant tumours in this region.

Oral tongue: It is the anterior two third of tongue. Four intrinsic and four extrinsic muscles form bulk of tongue. Extrinsic muscles are genioglossus, hyoglossus, styloglossus and palatoglossus. Intrinsic muscles are inferior and superior longitudinal, transverse and vertical muscle.

Retromolar trigone: This is the attached mucosa overlying the ascending ramus of the mandible from the level of the posterior surface of the last molar tooth and the apex superiorly, adjacent to the tuberosity of the maxilla.

Floor of mouth: It is the mucosal surface bordered by oral tongue medially and inferior alveolar ridge laterally and anteriorly. Posterior margin is anterior tonsillar pillar. The lingual frenulum divides the region into two oral spaces.

Buccal mucosa: From the vermilion it extends between posterior aspect of lip to the alveolar ridge medially and pterygomandibular raphe posteriorly.

Hard palate: maxillary alveolar ridge form the anterior lateral margin and soft palate as posterior border.[11]

Figure 1

Anatomy of oral cavity showing hard palate and dorsum of tongue

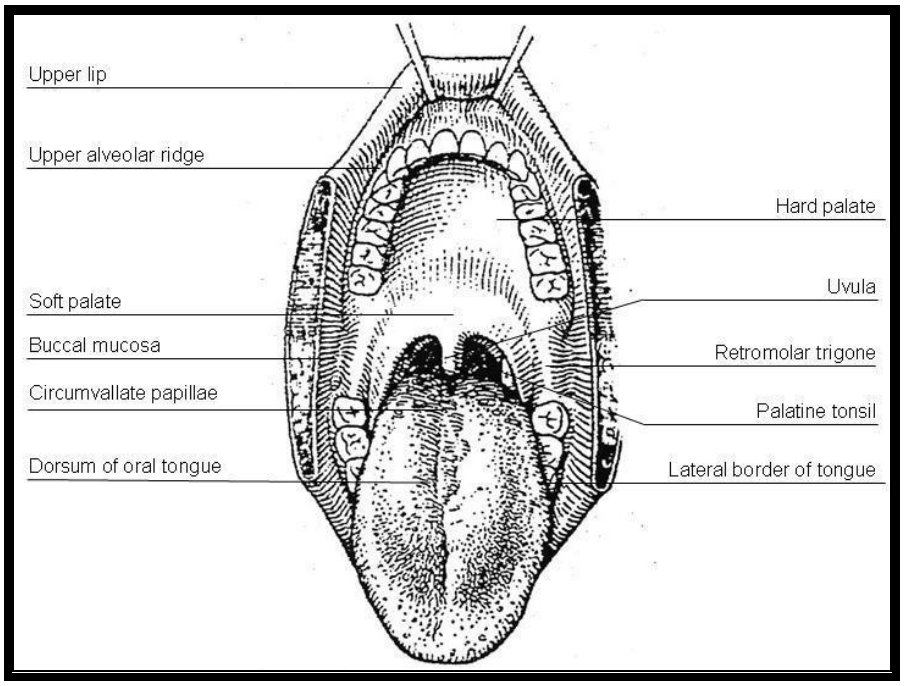
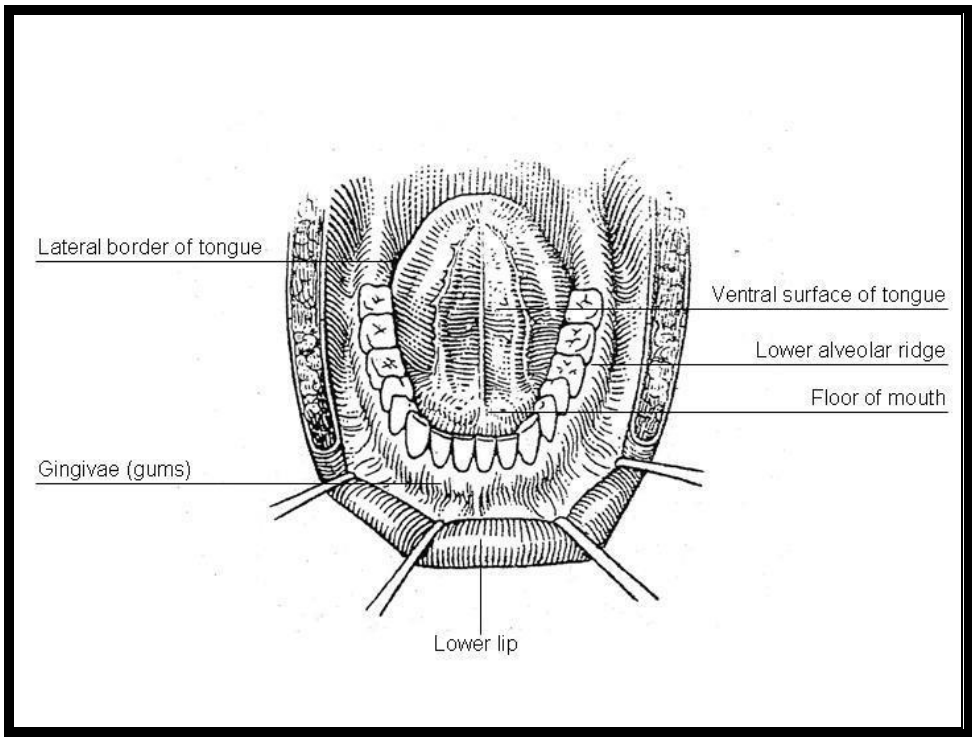


Figure 2

Anatomy of oral cavity showing ventral surface of tongue and floor of mouth



Epidemiology

Oral cancer is one of the most common malignancies in Southeast Asia, accounting for up to 30-40% of all malignancies in India.Oral cancer occurs most commonly in middle-aged and elderly individuals; however, recent evidence suggests these demographics may be changing.

Surveillance Epidemiology and End Results (SEER) data demonstrated an increase in the incidence of tongue cancer in young individuals (<40 years old), from 3% in 1973 to approximately 6% in 1993, and many of the affected individuals are without traditional risk factors. Additionally, research indicates that the traditional male predominance is less overt in young individuals with oral Squamous Cell Carcinoma (SCC). This trend is thought to be a reflection of the general acceptance of social habits such as chewing (tobacco,beetal nut), smoking and drinking by both sexes.

Risk Factors and Pathophysiology

Tobacco and alcohol independently can cause oral cancers. Heavy tobacco smokers have a 20-fold greater risk; heavy alcohol drinkers a 5-fold greater risk and those exposed to both have a 50-fold greater risk of oral cancer.

Tobacco in various forms like snuff and betel quid(a mixture of ingredients including betel leaf, areca nut, slaked lime, and tobacco, which is wrapped in a betel leaf and chewed), is carcinogenic . Alcoholic beverages may contain carcinogens or procarcinogens, including nitrosamine and urethane contaminants and ethanol. Ethanol is metabolized by alcohol dehydrogenase and, to some extent, by cytochrome P450 to acetaldehyde, which may be carcinogenic .[12]

There is little convincing evidence that mouthwash use, poor oral hygiene, or oral infections of viral origin Human Papilloma Virus (HPV 16 and 18) play an important role in the aetiology. Consuming fruit and vegetables may have a protective effect.

Patients who have had renal transplants have a higher incidence of cancer of the lip which may be due to immunosuppression.[13,14]

The tumors of smokers have a higher frequency of common genetic changes , such as p53 mutations, loss of heterozygosity at chromosomes 3p, 4p, 11q13, higher percentages of chromosomal microsatellite abnormalities.

Normal histology of oral mucosa:

Oral musoca is lined by non keratinizing stratified squamous epithelium.

Terminology and Definitions

The World Health Organization classifies oral precancerous/potentially malignant disorders into two general groups, as follows:[13,15]

- A precancerous lesion is "a morphologically altered tissue in which oral cancer is more likely to occur than its apparently normal counterpart." These precancerous lesions include leukoplakia, erythroplakia, and the palatal lesions of reverse smokers.
- A precancerous condition is "a generalized state associated with significantly increased risk of cancer." These precancerous conditions include submucous fibrosis, lichen planus, epidermolysis bullosa, and discoid lupus erythematous.

Pre malignant lesions:

Precancerous lesions:

Leukoplakia: a predominantly white, uniform, and flat lesion, not able to be scraped off, or can be reversed by the removal of irritants,or ascribed to another disease entity. Various clinical types of leukoplakia are homogeneous, nodular, verrucuous, erosive forms

Subtype :

- homogenous white patch with a variable appearance smooth or wrinkled.

- Speckled or nodular : erythematous base with a white patch or nodular excrescences
- Leukoplakia simplex:white homogenous keratinized lesion,slightly elevated
- Leukoplakia verrucosa: white verrucous lesion with velvety/wrinkled surface
- Leukoplakia erosive: white lesion with erythematous areas, erosions,fissures

Proliferative verrucous leukoplakia : a series of irregular white patches or plaques that progress slowly across the oral mucosa membranes with nearly a 100% risk of malignant transformation and a high risk of recurrence after removal.

Hairy leukoplakia : was originally thought to be associated with HPV but is now believed to be due to Epstein Bar Virus(EBV). This lesion develops in patients with Human Immunodeficency Virus(HIV) infection and characteristically located along the lateral edges of the tongue. Microscopically it shows parakeratosis , acanthosis intranuclear inclusions,keratinocytes,associated with ballooned or ground glass cytoplasm.

The term **leukokeratosis** was used for patches with a histologically benign appearance.

The white colour of leukoplakia is a result of hydration of a thickened horny layer. 80% of lesions are benign. Histologically , hyperkeratosis ,parakeratotic thickening of horny layer acanthosis , chronic inflammatory infiltrate are seen.

Erythroplakia: a velvety red lesion with imprecise borders that could not be diagnosed as any other lesion. Histologically ,in contrast to leukoplakia, invariably show nuclear atypicality,in situ anaplasia in half of cases and invasive carcinoma in other half. The red appearance is explained by the absence of the nuclear surface covering of orthokeratin or parakeratin. It has a malignant potential of 30-50%.

Palatal lesion of reverse smokers

The palatal lesion of reverse smokers is unique to individuals who place the lit end of a cigarette inside the mouth. The resulting palatal lesion may appear clinically as a red, white, melanotic patch or papule.

Precancerous conditions:

Oral submucous fibrosis(OSF) : is a chronic progressive condition that appears clinically as whitish mucosa lacking elasticity. Epithelial dysplasia has been described in 7-26% of OSF tissues, and long-term studies suggest a malignant transformation rate in approximately 7% of these lesions.

Lichen planus: a predominantly red, irregular erosion or ulceration associated with a reticular form, especially in the peripheral region of the lesion and with pseudomembranes covering the ulcerated areas.

Reticular lichen planus: a predominantly white lesion with intertwining lines or striae that confer a lacy or annular appearance .

Lichenoid dysplasia is a premalignant form of lichen planus characterized by cytological alterations of dysplasia.

Discoid lupus erythematous, and epidermolysis bullosa

Although classified as potentially malignant conditions, the data regarding progression to malignancy for these conditions is controversial. Because of the difficulty in classifying and clinically distinguishing the varied lesions associated with these conditions, the potential for malignant transformation remains unclear.

Nicotine stomatitis :clinically , it is seen as white palatal proliferation with central red areas representing inflamed or obstructed minor salivary gland ducts.

Histologically : it shows hyperkeratinized epithelium and marked squamous metaplasia with deep rete ridge penetration into the connective tissue lamina propria

with diffuse inflammation that frequently surrounds small ducts with associated minor salivary glands.

Superficial ulcerations suspicious of malignancy: localized, superficial lesions without invasion or loss of mobility of neighboring chronic tissues that do not heal after local treatment.[4,16,17]

Squamous dysplasia and carcinoma in situ

Dysplasia refers to abnormal epithelial growth that is characterized by features of cytologic,maturational and architectural changes. The World Health Organization(WHO) collaborating center for oral precancerous lesions has defined a battery of histological features that are characteristic of squamous oral dysplasia. [18]

Histologic features of squamous dysplasia:

*loss of polarity of the basal cells

*presence of more than one layer of cells having a basaloid appearance

*an increased nuclear –to-cytoplasmic ratio

*drop shaped rete processes

*irregular number of mitotic figures

*presence of mitotic figures in the superficial half of the epithelium

*cellular pleomorphism

*nuclear pleomorphism

*enlarged nucleoli

*reduction of cellular cohesion

*keratinization of single cells or groups of cells in the prickle layer

Oral epithelial dysplasia is usually classified as mild, moderate and severe.

In mild dysplasia, the severity of atypical change is minimal, the most prominent features may include only basal layer hyperplasia,loss of cellular polarity atypical mitosis. The surface layer is usually hyperkeratinized, so the lesion appears white clinically. As the atypical cytological changes increase to include a greater frequency of altered nuclear cytoplasmic ratio, dyskeratosis, basal layer hyperchromatosis, mitosis, the degree of severity of dysplasia increases proportionately.

Carcinoma in situ (intraepithelial carcinoma)

The diagnosis of carcinoma insitu of the oral mucosa should be based on rigid histologic criteria, however, the distinction is often arbitrary. With carcinoma insitu one must identify an intact basement membrane and top to bottom or through and through epithelial dysplasia.

Squamous cell carcinoma:

Grossly ,SCC of oral cavity can present as an ulcer , an alteration of mucosal color or a tumor mass. Cut section has a gray white glistening appearance.

Histology : SCC is characterized by a proliferation of sheets, nests, cords and neoplastic islands of epithelium that penetrate into the supporting connective tissue lamina propria and submucosa.

The neoplasm is categorized according to Brooder s Classification into:

Well Differentiated Squamous Cell Carcinoma (WDSCC)

Moderately Differentiated Squamous Cell Carcinoma (MDSCC)

Poorly Differentiated Squamous Cell Carcinoma (PDSCC)

Undifferentiated Squamous Cell Carcinoma (UDSCC)

WDSCC: The neoplastic cells have striking similarity to the cells of normal squamous epithelium. The cells are generally large with vesicular to oval nuclei and eosinophilic cytoplasm, intracellular bridging is seen. Keratin pearl formation is quite prominent and individual cell keratinization is hallmark of this disease.

MDSCC: hyperchromatism, pleomorphism are prominent. Atypical mitoses is increased. Keratin pearl formation and individual cell keratinization is decreased.

PDSCC: very little evidence that tumor is of squamous origin. Pleomorphism and atypical mitoses are prominent.

UDSCC (non keratinizing SCC): Tumor cells resemble histiocytes, atypical lymphocytes or spindled fibroblasts. Stroma shows desmoplastic fibrosis and chronic inflammatory infiltrate.

Staging and prognosis:

Patient prognosis for SCC of the oral cavity is determined by evaluating the initial tumor size and the extent of metastasis to either regional lymph node or distant organs.

TNM staging system of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) for lip and oral cavity cancer , 7th edition.

The Tumor Node Metastasis (TNM) system for the clinical staging of SCC is the most commonly used method of defining prognosis[17]:

T stage for oral cancer

Primary Tumor

TX Cannot be assessed

T0 No evidence of primary tumor

Tis Carcinoma in situ

T1 Tumor 2 cm or less in greatest dimension

T2 Tumor more than 2 cm but not more than 4 cm in greatest dimension

T3 Tumor more than 4 cm in greatest dimension

T4a Moderately advanced local disease. Lip: Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face, ie, chin or nose

Oral cavity: Tumor invades adjacent structures (eg, through cortical bone [mandible, maxilla], into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face)

T4b Very advanced local disease. Tumor invades masticator space, pterygoid plates, or skull base, and/or encases internal carotid artery

N stage for oral cancer

NX Cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension

N2a Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension

N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension

N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension

N3 Metastasis in a lymph node more than 6 cm in greatest dimension

M stage for oral cancer

M0: No metastasis are present.

M1: The cancer has spread to distal organs (organs located far from the origin point where the cancer had developed initially).

Based on the NTM system, the oral cancer is classified in four stages:

Stage I: (T1, N0, M0)

In this stage, the cancer is confined to tissue where it initially occurred, and the tumor is not larger than 2 cm.

Stage II: (T2, N0, M0)

In this stage, the tumor is no larger than 4 cm.

Stage III: This stage includes two substages:

Stage IIIA: (T3, N0, M0)

In this stage, the tumor is larger than 4 cm, but no lymphatic nodes or metastasis are present.

Stage IIIB: (T1, T2, T3, N1, M0)

In this stage, the tumor size is either less than 2 cm, under 4 cm, and 4 cm or over, but the cancer has affected one homolateral lymphatic node.

Stage IV: This stage includes three substages:

Stage IVA: (T4, N0, M0)

In this stage, the tumor is larger than 4 cm, and it has deeply invaded the muscle, bone, or other adjacent structures

Stage IVB: (Any T, N2 or N3, M0)

In this stage, the tumor can have several sizes (1) less then 2 cm, (2) less or more than 4 cm, (3) more than 4 cm but it has deeply invaded the muscle, bone, or other adjacent structures, or the cancer has spread to several homolateral or bilateral lymphatic nodes.

Stage IVC: (Any T, any N, any M)

In this stage, there are several situations which include the tumors having different

sizes (between 2 and more than 4 cm), the cancer is present in the homolateral or bilateral lymphatic nodes and in other organs within the body.

Histological features of prognostic significance:

Two apparent significant histologic features of oral SCC are predictive of patient outcome:

1. The pattern of tumor invasion within the supporting collagenous stroma.

2. The depth of tumor invasion into that collagenous stroma.[15,16,17,18,19]

Brush biopsy /cytology:

The oral mucosa is made of non keratinizing stratified squamous epithelium.

The normal cytology of oral mucosa shows 4 types of cells : superficial squamous cell, intermediate squamous cell,parabasal cell and basal cell.

Superficial squamous cells: these are large polygonal cells (diameter of 35-45 microns), having flat delicate , transparent cytoplasm and small dark pyknotic nuclei of 4 micron in diameter. In Papanicalaou stains, the cytoplasm stains delicate pink due to affinity of cytoplasm for acid dyes such as eosin. The nucleus stains blue due to affinity for basophilic dyes.

Intermediate squamous cells: smaller than superficial squamous cell. Their cytoplasm is usually basophilc. The nucleus is spherical or oval measures 8 microns in diameter.

Parabasal cells : measures 12-30 microns in diameter. The nuclei are vesicular and measures 8 micron in diameter. The nuclei are usually bland and homogenous.

Basal cells :small cells having scanty cytoplasm and nucleus measures 8 microns in diameter. These cells are protected and are practically never seen in smears. These should not be confused with small cancer cells of similar size and configuration.

Abnormal cells in brush biopsy/cytology:

Cancer cells can be recognized by their marked nuclear abnormalities and are classified according to their cytoplasmic features. The recognition of precursor lesions was based on the concept of dyskaryosis (from greek, dys=abnormal and karion=nucleus) introduced by Papanicolaou in 1949. Dyskaryotic cells differ from cancer cells by their well differenciated mature cytoplasm and lesser although variable levels of nuclear abnormalities. The term dysplastic cells are used currently.

Nuclear enlargement or karyomegaly :

The term karyomegaly (from greek , karyon = nucleus and megalos =large) was proposed by Papanicolaou in 1949 to describe enlargement of nuclei occurring in superficial squamous cell, intermediate squamous cell, parabasal cells with morphologically normal cytoplasm. The nuclei may be transparent, but are often opaque and hyperchromatic.

Karyomegaly represents an abnormality of the nuclear structure. The nuclei is enlarged two and a half to three times above size of normal nuclei.

Dysplastic cells :

The nuclei show karyomegaly and an abnormal chromatin texture in the form of coarse clumping of chromatin, thickening of nuclear membrane and nuclear creases or folds. The nuclei also display an irregular contour in the form of small indentations, notches or spikes visible under the high power of the microscope.

Cancer cells :

Cancer cells, whether derived from invasive cancer or high grade precursor lesions,usually display significant nuclear and cytoplasmic abnormalities

Cancer cells of squamous origin:

Squamous cancer cells are characterized by an extraordinary variety of cell shapes and formation of abundant keratin. The nuclear sizes are variable. They may be hyperchromatic, coarsely granular and of irregular shape. In few cells, the nuclei may become pale and in final stages of keratinization, may disappear altogether, submerged by overgrowth of keratin. Such anucleated squames of bizarre shapes are diagnostically as important as nucleated squamous cancer cells.

Tadpole cells (cells with a tail or caudate cells) are uncommon cells observed mainly in invasive squamous cancer and in high grade squamous precursor lesions. Spindly squamous cells are elongated and needle shaped and vary in length from 10-40 microns. Squamous pearls are concentrically arranged clusters of squamous cancer cells. Cancerous pearl have enlarged and hyperchromatic nuclei.

Undifferentiated cancer cells :

Vary in size from small to gigantic forms. Usually accompanied by debris, fresh and lysed or fibrinated blood, and leukocytes, all consisting evidence of necrosis and inflammation accompanying advancing or advanced cancer referred to as "cancer diathesis" [2,20]

Toluidine blue

Supravital staining means staining the live RNA or DNA of the cell.

Several studies have shown that supravital stains such as toluidine blue ,methylene blue and Lugol iodine solution have improved diagnostic accuracy of clinical examinations.[21]

Toulidine blue (TB) is a cationic metachromatic vital dye that binds to sulphates, phosphates and carboxylates, hence bind to tissues undergoing rapid cell division such as inflammatory, regenerative and neoplastic tissue.[22] This dye is used in cell

culture to separate live intact cells from with altered membrane. The latter results in leaky cell membrane, allowing the dye to enter and bind to the phosphate groups on the nuleic acids, which stains the nucleus dark blue in 60 seconds with a concentration as low as 1% dye. This dye is suggested for use as an adjunct diagnostic test for the early detection of epithelial dysplasis and SCC of the oral cavity,esophagus and cervix .[23,24]

Toluidine blue (TB) is partially soluble both in water and in alcohol. It is used as a vital stain to highlight potentially malignant oral lesions (PML) and identify early lesions which could be missed on clinical examination. Moreover it can outline the full extent of the dysplastic epithelium or carcinoma when excisions are planned, can detect multicentric or metachronous second tumors and can help the follow-up of patients with oral cancer. It is useful to obtain the biopsy sample from the suspicious site.[4]

Toluidine blue, a basic metachromatic nuclear stain that stains nuclear material of malignant lesions but not normal mucosa, has been established as a useful agent for identifying malignant changes of the squamous mucosa.[25]

Although useful as an adjunct to clinical examination, the specificity of TB staining is limited because cells undergoing inflammatory changes and benign hyperplasia may also retain dye leading to false-positive results.

Brush Biopsy (BB)

Available cytologic techniques have remained relatively unchanged for many decades. In the oral cavity, these have been of limited use due to the superficial nature of the cells collected and the keratin layer that is often present. As a result, deeper epithelial abnormalities often went undetected. The transepithelial brush biopsy

technique has evolved so that superficial and deep regions of epithelium can be harvested successfully.

BB acts as a screening procedure for the early detection of oral cancers. It is a non invasive technique and can be used in patients who do not warrant immediate biopsy. Dysplastic and cancerous cells tend to have fewer and weaker connections to each other and to their neighboring normal cells in the surrounding tissue, therefore, tend to "slough off" or exfoliate easily be collected from the surface of the lesion. A sample of these cells applied to a microscope slide will often contain abnormalities if harvested from a dysplastic or cancerous lesion.[26]

Unlike cervical cytology, which has been shown to be an accurate method of detecting dysplasia, the examination of oral cytology specimens obtained by brushing of the oral mucosa with a spatula or cytobrush results in an unacceptably high number of errors.

The use of oral cytology can be a means to accelerate biopsy of these clinically harmless appearing cancers that would have otherwise been neglected.[27]

MATERIALS AND METHODS

MATERIALS AND METHODS

Source of data (sample):

Our study is a prospective study. A total of 172 patients with suspicious oral lesions who attended the outpatient clinics of Otorhinolarynglogy and Dental surgery at R. L. Jalappa hospital and Research, Centre attached to Sri Devaraj Urs Medical college, Tamaka, Kolar, from time interval of September 2008 to March 2010 were screened with in vivo toluidine blue staining oral brush biopsy and scalpel biopsy . The ethical clearance has been obtained from the Ethics Clearance Committee of Sri Devaraj Urs Medical College, Kolar.

Inclusion Criteria:

All patients with clinically suspicious oral precancerous and cancerous lesions.

Exclusion Criteria:

Patients with any medical problem and dental conditions, such as Orthodontic or other fixed prostheses which may interfere with the examination.

Patients who had been previously treated for cancerous condition by surgery or irradiation.

Method of collection of data:

Patients with suspicious premalignant or malignant lesions of the oral cavity, irrespective of site, stage and sex were selected. On local examination ,the intra oral location, lesion size, extent of local infiltration, oral hygiene and cervical lymph node enlargement were assessed .

Materials required for the study are given below and shown in figure 3:

*Sterile gloves

*1% toulidine blue

*1% acetic acid

*Cotton swab

*Tooth brush

*Slides

*Biofix spray

*Rapid PAP Staining kit

In vivo toluidine blue staining:

***1%** acqueous toluidine blue was applied to the suspicious lesion for 30 seconds,

*Followed by tap water or normal saline rinse.

*Then rinsed by 1 % acetic acid for 30 seconds to reduce the back ground staining.

*The procedure of staining is shown in figure 4

Oral brush biopsy:

Using a small hard tooth brush as shown in (figure 5), a transepithelial brush biopsy was taken. Removed cells was transferred to a glass slide by distributing the obtained material evenly over the glass surface as shown in (figure 6). Slides were flooded with fixative as shown in(figure 7), the cellular sample on the slide were stained with Papanicolaou stain and hematoxylin and eosin stain and examined by light microscopy.

Interpretation of brush biopsy was done as follows:

*Assessment of adequacy of smear.

*Smears analysed for enlarged nuclei ,variation in nuclear /cytoplasmic ratio, number of nuclei, hyperchromatism, discrepancy in maturation .

*smear was categorized as benign, suspicious for malignancy or malignant lesions.

Scalpel biopsy:

*The biopsy obtained was fixed in 10% formalin saline ,processed and thin sections were made.

*They were stained with hematoxylin and eosin.

*The sections were examined under light microscope for confirmation of diagnosis which is the gold standard for the diagnosis.

Statistical analysis was done using statistical software SPSS version 16. Chi square test was used to test the test of significance and a p value of <0.05 was taken as statistically significant. To test the diagnostic accuracy of the screening tests sensitivity,specificity and predictive value were calculated.

Figure 3

Materials for the study

Figure 4
The technique of staining of toulidine blue

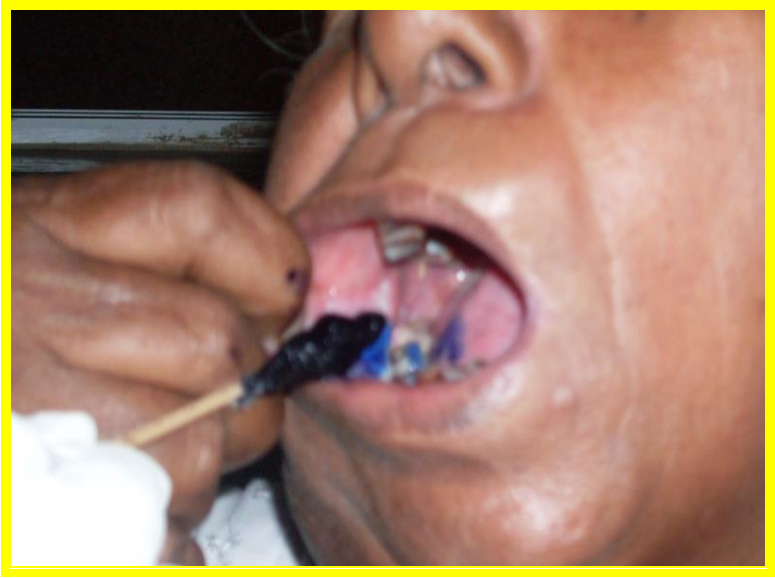


Figure 5
Brush biopsy procedure

Figure 6
The procedure of smearing on slide

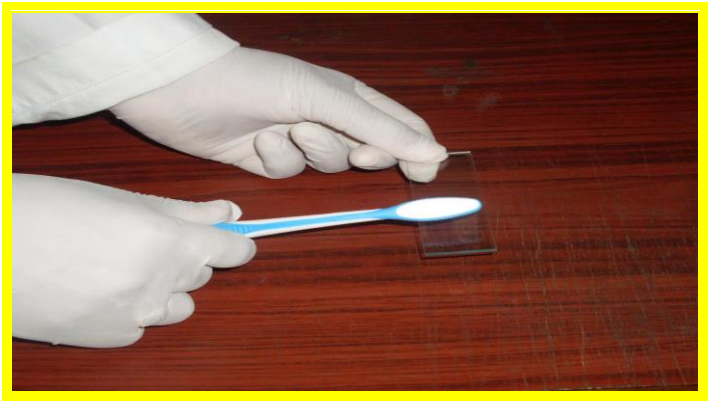


Figure 7
The procedure of fixing the smear

RESULTS

RESULTS

This is a prospective study of 172 patients who came to the hospital with premalignant/malignant oral lesions. Screening techniques of supravital staining with toluidine blue application and Brush biopsy were done for all these patients. All were followed up with histopathological examination which is considered as gold standard for the diagnosis.

Age distribution :

The age distribution is shown in table 1 and figure 8

The age of the patients varied from 18 years to 90 years. Mean age was 55 years. The maximum numbers of cases (69.7%) were seen in the age group over 46 years. The youngest patient was of 18 years and the oldest patient was of 90 years .

Table 1

Distribution of cases in different age groups.

Age group	Number of cases	%
<26	1	0.6
26-35	16	9.3
36-45	35	20.3
46-55	42	24.4
>55	78	45.3
Total	172	100.0

Sex distribution :

The sex distribution can be seen in table 2 and figure 9.

Out of 172 cases predominantly 127 cases were females and 45 were males.

Table 2

Sex distribution

Sex	No.of Patients (n =172)	%
Females	127	73.8
Males	45	26.2
Total	172	100.0

Clinical presentations of patients:

The various complaints by patients are mentioned in table 3

Most of the patients had pain (73%) and very few had trismus 5%

Table 3

Clinical presentations

Complaint	**No.of Patients (n =172)**	**%**
Pain	126	73.25
Halitosis	119	69.2
Trismus	10	5.81
Lymphnode enlargement	112	65.1
Weight loss	103	59.9

Etiological risk factors:

The exposure to etiological risk factors is depicted in table 4.

Of 172 cases maximum of (84.3%) had a habit of areca nut /tobacco chewing .

Table 4

Etiological risk factors

Etiology	**No.of Patients**	**% (n =172)**
Smoking	50	29.06
Alcohol	48	27.9
Areca nut/tobacco	145	84.3

Combination of risk factors

Combination of risk factors for oral cancers are shown in table 5

Table 5

Combination of risk factors

Risk factors	**Number of cases**	**% (n =172)**
Smoking +alcohol+tobacco	21	12.2
Smoking+pan	15	8.72
Alcohol +pan	23	13.4

Duration of exposure to carcinogens :

The duration of exposure to carcinogens is shown in table 6 and figure 10

Of 172 patients 140 had exposure to carcinogens. Majority of patients 57% developed cancer between 11-30 years of exposure. Only 11 cases had an exposure of below 10 years.

Table 6
Duration of exposure to carcinogens

Duration of exposure (years)	**No.of Patients (140)**	**% n =172**
0-10	11	6.3
11-20	41	23.8
21-30	57	33.1
31-40	23	13.3
41-50	7	4.0
51-60	1	0.5

Site of the lesions :

The various site of lesions is mentioned in table 7 and figure 11.

Of 172 cases maximum number of cases were seen in buccal mucosa (62.8%), next highest was tongue (12.8%) and least incidence were in lip (1.2%)

Table 7

Site of the lesions

Site	**Number of cases**	%
Buccal mucosa	108	62.8
Floor of mouth	5	2.9
Hard palate	10	5.8
Lip	2	1.2
Tongue anterior 2/3rd	22	12.8
Upper alveolus	9	5.2
Lower alveolus	8	4.6
Retro molar trigone	8	4.7
Total	172	100

Size of the lesions:

The distribution of size of the lesions of premalignant and malignant cases is depicted in table 8 and table 9 respectively.

Among malignant lesions most of the cases 33% were less than 2 cm.

Among the premalignant lesions maximum cases 15% were of less than 2cm.

Table 8

Size of the lesions (malignant lesions)

Size in cm	**Number of cases**	% (n=172)
<2	57	33.13
2-4	48	27.9
>4	21	12.2

Table 9

Size of the lesions (premalignant lesions)

Size in cm	Number of cases	% (n=172)
<2	26	15.1
2-4	14	8.13
>4	6	3.4

Type of the lesion :

The various morphological types of lesions are mentioned in table 10.

Most of the lesions 57.6 % were ulceroproliferative

Table 10

Type of the lesion

Type	Number of cases	%
Plaque	32	18.6
Ulcerative	41	23.9
Ulceroproliferative	99	57.6
Total	172	100

Clinical diagnosis :

The various clinical diagnosis of the lesions are mentioned in table 11

Majority of the cases were suspected as carcinoma (74.41%) and only one case of submucosal fibrosis was diagnosed.

Table 11
Clinical diagnosis

Clinical diagnosis	Number of cases	%
Carcinoma	128	74.41
Leukoplakia	35	20.34
Erythroplakia	8	4.65
Submucosal fibrosis	1	0.58
Total	172	100

Supravital staining of oral lesions with toulidine blue (TB):

The pattern of staining is shown in table 12 and figure 12

The oral lesions were stained with TB. Based on the amount of retention of the dye the oral lesions were categorized into strongly positive, weakly positive and negative which are depicted in table 12 and figure 12**.**

Table 12

Toulidine blue staining of oral lesions:

Toulidine blue	Number of cases	%
Strongly positive	124	72
Weakly positive	32	18.6
Negative	16	9.4
Total	172	100

Table 13
Strong positive cases with staining of toulidine blue

Lesions	Number of cases (124)	% (n=172)
Carcinomas	116	67.4
Leukoplakia	4	2.32
Erythroplakia	2	1.16
Chronic ulcer	2	1.16

Table 14
Weak positive cases with staining of toulidine blue

Lesions	Number of cases (32)	% (n=172)
Mucoepidermoid carcinoma	2	1.16
Microinvasive carcinoma	1	0.58
Carcinoma in situ	1	0.58
Dysplasia	4	2.32
Leukoplakia	15	8.7
Erythroplakia	6	3.4
Dyskeratosis	3	1.74

Table 15
Negative cases with staining of toulidine blue

Lesions	Number of cases	%
Mucoepidermoid carcinoma	2	1.16
Leukoplakia	6	3.48
Submucosal fibrosis	1	0.58
Squamous papilloma	2	1.16
Kimura s disease	1	0.58
Lobular angioma	1	0.58
Pseudoepithelomatous hyperplasia	2	1.16
Candidiasis	1	0.58

Brush biopsy results :

The results of cytological examination of brush biopsy smears were categorized as malignant, suspicious for malignancy and benign lesions as depicted in table 16 and figure 13.

Table 16
Brush biopsy results

Brush biopsy	Number of cases	%
Malignant	119	69.1
Suspicious for malignancy	16	9.3
Benign	37	21.5
Total	172	100

The comparison of Brush biopsy malignant smears with final histopathology is shown in table 17.

Table 17
The lesions diagnosed as malignancy on cytology

Histopathological diagnosis	Number of cases (119)	% (n=172)
Carcinomas	115	66.8
Dyskeratosis	3	1.74
Chronic ulcer	1	0.58

The comparison of Brush biopsy suspicious for malignancy smears with final histopathology is shown in table 18.

Table 18
The lesions diagnosed as suspicious for malignancy on cytology

Histopathological diagnosis	Number of cases (16)	% (n=172)
Verrucous carcinoma	3	1.16
Mucoepidermoid carcinoma	2	1.16
Carcinoma in situ	1	0.58
Dysplasia	4	2.32
Pseudoepithelomatous hyperplasia	2	1.16
Erythroplakia	3	1.74
Chronic ulcer	1	0.58

The comparison of Brush biopsy of benign smears with final histopathology is shown in table 19.

Table 19
The lesions diagnosed as benign on cytology

Histopathological diagnosis	**Number of cases (37)**	**% (n=172)**
Microinvasive carcinoma	1	0.58
Leukoplakia	25	14.5
Erythroplakia	5	2.9
Squamous papilloma	2	1.16
Submucosal fibrosis	1	0.58
Kimura s disease	1	0.58
Lobular angioma	1	0.58
Candidiasis	1	0.58

Wedge biopsy (histopathology) diagnosis:

This is considered as gold standard. Of 172 cases 126 cases turned out to be malignant and 46 cases were benign as shown in table 20

Table 20

Histopathology diagnosis of oral lesions

Histopathological diagnosis	**Number of cases**	**%**
Malignant	126	73.25
Benign	46	26.74
Total	172	100

The malignant cases are mentioned in table 21 and figure 14 and benign cases in table 22 and figure 15.

Majority of cases among malignant were diagnosed as Well Differentiated Squamous Cell Carcinoma (41.22%) and single cases of microinvasive carcinoma and carcinoma in situ.

Table 21

Histopathology of Malignant lesions

Cancer/dysplasia	**Number of cases**	**%** **n = 172**
Well Differentiated Squamous Cell Carcinoma	71	41.22
Moderately Differentiated Squamous Cell Carcinoma	40	23.25
Poorly Differentiated Squamous Cell Carcinoma	2	1.16
Verrucous carcinoma	3	1.74
Mucoepidermoid carcinoma	4	2.32
Microinvasive carcinoma	1	0.58
Carcinoma in situ	1	0.58
Dysplasia	4	2.32
Total	126	73.25

Among benign lesions leukoplakia were maximum and single case each of Kimura s disease,lobular angioma,candidiasis,submucosal fibrosis were seen.

Among the 46 benign lesions as diagnosed by histopathology report , leukoplakia, erythroplakia, chronic ulcer, and submucosal fibrosis though benign were considered as premalignant lesions.

The rest of the cases squamous papilloma, Kimura s disease, lobular angioma, dyskeratosis, pseudoepithelomatous hyperplasia and candidiasis were considered as truly benign lesions**.**

Table 22
Benign lesions

Benign lesions	**Number of cases**	**% of n = 172**
Leukoplakia	25	14.53
Erythroplakia	8	4.65
Chronic ulcer	2	1.16
Submucosal fibrosis	1	0.58
Squamous papilloma	2	1.16
Kimura s disease	1	0.58
Lobular angioma	1	0.58
Dyskeratosis	3	1.74
Pseudoepithelomatous hyperplasia	2	1.16
Candidiasis	1	0.58
Total	46	26.74

Analysis of data

After screening of 172 cases with 1 % toulidine blue application and brush biopsy ,all cases were subjected to wedge biopsy and Histopathological examination.

The histopathological examination is considered as the gold standard and all screening tests i.e clinical diagnosis, toulidine blue and brush biopsy were compared with it for analysis of results.

Clinical diagnosis in comparison with histopathological diagnosis:
The comparison is shown in table 23.

Table 23
Clinical diagnosis in comparison with histopathological diagnosis:

Clinical diagnosis	Histopathology malignant	Histopathology benign	Total
Malignant	118	10	128
Benign	8	36	44
Total	126	46	172

False positives 10 cases

False negatives 8 cases

Comparison of toulidine blue staining with histopathological diagnosis:

Supravital stain Toulidine blue screening for malignant lesions is shown in table 24

The weak positive cases of TB is considered as negative for statistical analysis.

Table 24

Comparison of toulidine blue staining with histopathological diagnosis

TB staining	Histopathology malignant	Histopathology Benign lesions	Total
Positive(strongly positives)	116	8	124
Negative(weakly positives and negatives)	10	38	48
Total	126	46	172

Sensitivity of TB screening for malignant lesions=92.06%, Specificity =82.60%
Positive predictive value=93.54%, Negative predictive value=79.16%
False negatives=7.9%, False positives=17.39%

Comparison of brush biopsy and histopathological diagnosis:

Brush biopsy screening for malignant lesions

The comparison is depicted in table 25

Lesions suspicious for malignancy were considered as negative for statistical analysis.

Table 25

Comparison of brush biopsy and histopathological diagnosis

Brush biopsy screening	**Histopathology malignant**	**Histopathology benign lesions**	**Total**
Positive	115	4	119
Negative	11	42	53
Total	126	46	172

Sensitivity of brush biopsy screening for malignant lesions=91.26%

Specificity =91.30%

Positive predictive value=96.63%, Negative predictive value=79.24%

False negatives=8.7% , False positives=8.6%

Combined Toulidine Blue and Brush Biopsy screening for malignant lesions:

The comparison is shown in table 26

Table 26

Combined TB and BB screening for malignant lesions

Combined TB and BB screening	**Histopathology malignant**	**Histopathology Benign lesions**	**Total**
Positive	118	2	120
Negative	8	44	52
Total	126	46	172

Sensitivity of combined toulidine blue and brush biopsy screening for malignant lesions=93.65%, Specificity =95.65%

Positive predictive value=99.15%, Negative predictive value=84.61%

False negatives=6.3%, False positives=4.3%

Comparison of toulidine blue staining of premalignant lesions with histopathology:

The comparison is shown in table 27

Table 27

Comparison of toulidine blue staining of premalignant lesions with histopathology

TB screening	Histopathology Premalignant lesions	Histopathology Benign lesions	Total
Positive	22	2	24
Negative	14	8	22
Total	36	10	46

Sensitivity of TB screening for premalignant lesions=61.1%

Specificity =80%

Positive predictive value=91.6%, Negative predictive value=36.33%

False negatives=38.8%, False positives=20%

Brush biopsy screening for premalignant lesions

The comparison is shown in table 28

Table 28

Brush biopsy screening for premalignant lesions

Brush biopsy screening	Histopathology Premalignant lesions	histopathology Benign lesions	Total
Positive	30	1	31
Negative	6	9	15
Total	36	10	46

Sensitivity of brush biopsy screening for premalignant lesions=83%

Specificity =90%

Positive predictive value=96.7%, Negative predictive value=60%

False negatives=16.66%, False positives=10%

Combined TB and BB screening for premalignant lesions:

The comparison is shown in table 29

Table 29

Combined TB and BB screening for premalignant lesions:

Combined TB and BB screening	**Histopathology Premalignant lesions**	**Histopathology Benign lesions**	**Total**
Positive	32	1	33
Negative	4	9	13
Total	36	10	46

Sensitivity of combined toulidine blue and brush biopsy screening for premalignant lesions=88.88%, Specificity =90%

Positive predictive value=96.96%, Negative predictive value=69.23%

False negatives=11.11%, False positives=10%

Statistical analysis of invivo Toluidine blue staining , brush biopsy and combined TB and BB on premalignant and malignant lesions:

The comparison of various statistical parameters are shown in table 30

Table 30

Statistical analysis	**Premalignant lesions**			**Malignant lesions**		
	TB	BB	TB+BB	TB	BB	TB+BB
Sensitivity (%)	61	83	88	92	91	93
Specificitity(%)	80	90	90	82	91	95
Positive predictive value (%)	91	96	96	93	96	99
Negative predictive value (%)	36	60	69	79	79	84

Figure 8

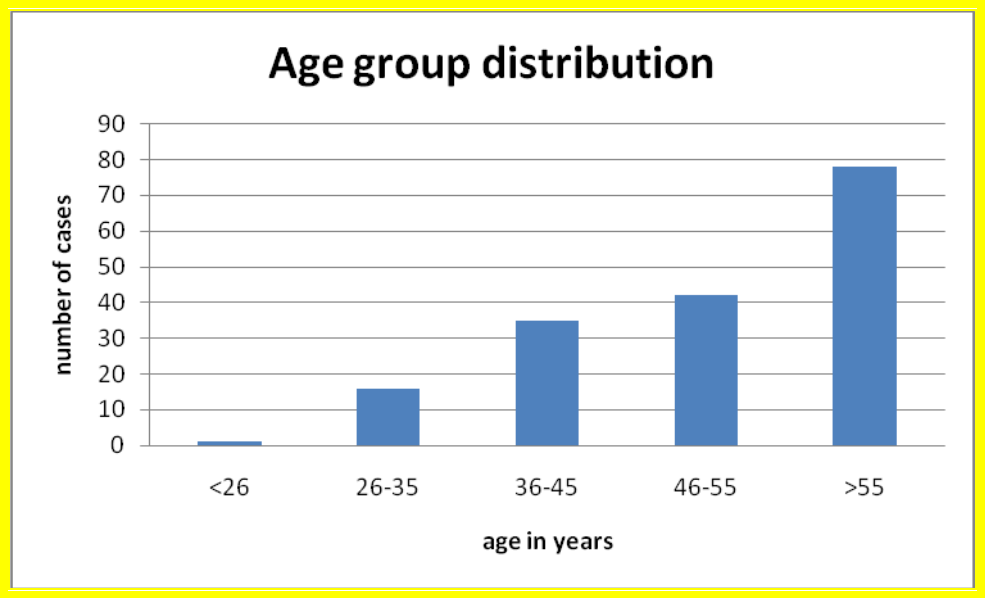


Figure 9

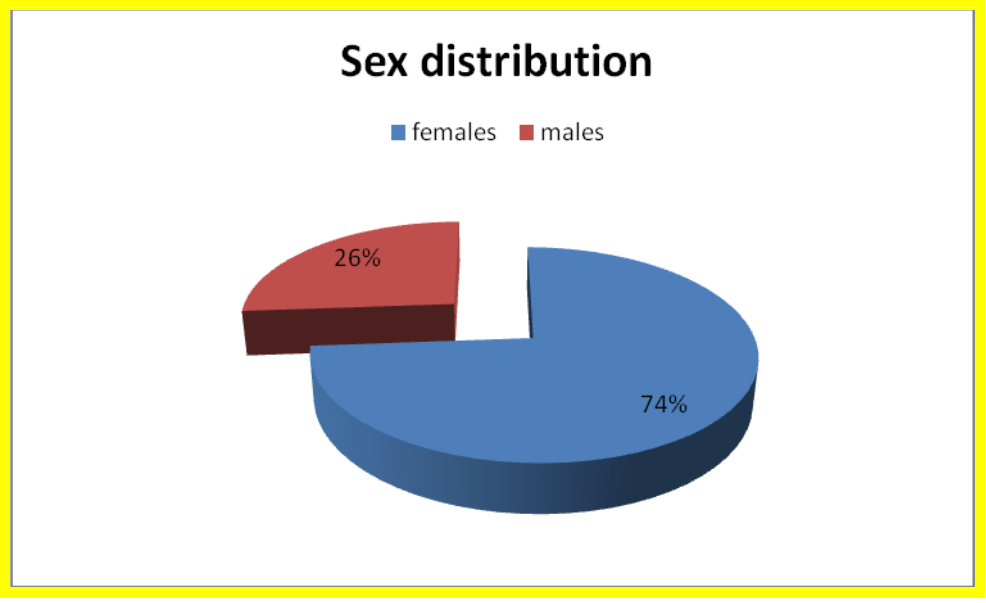


Figure 10

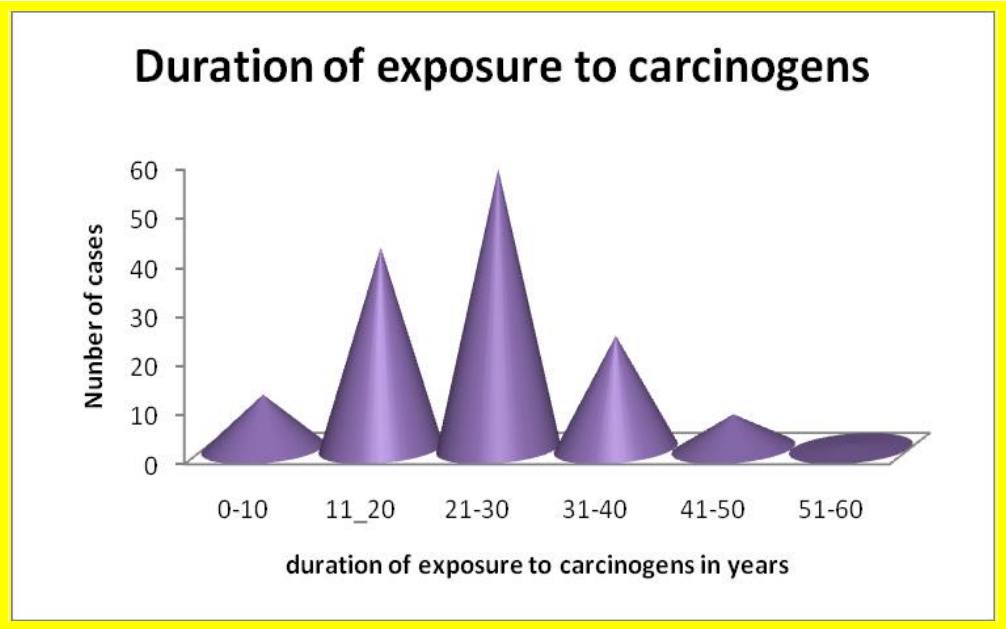


Figure 11

Site of the lesions

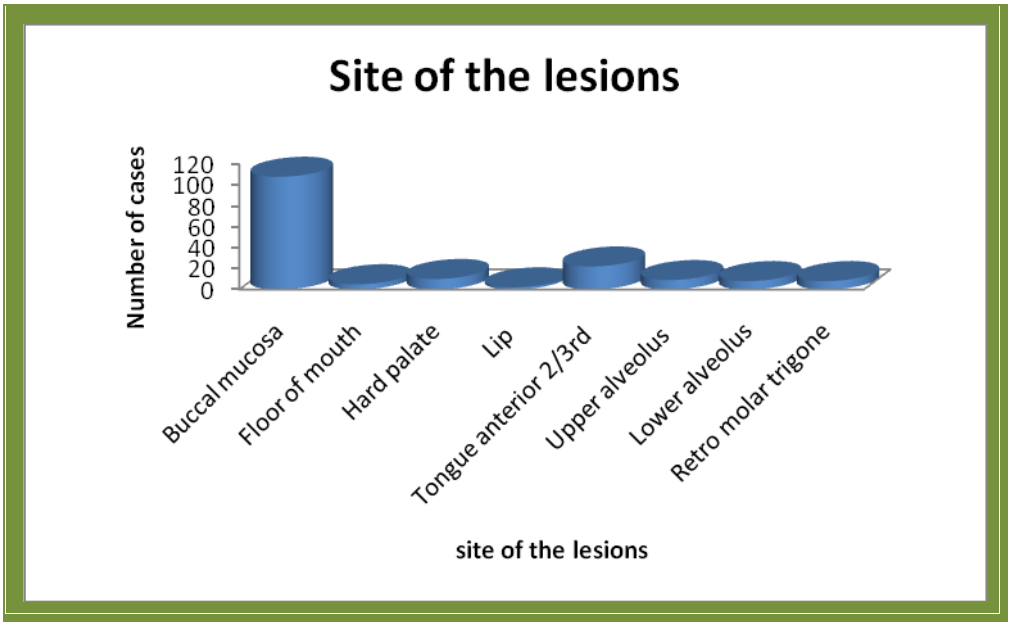


Figure 12
Staining of the lesions with Toulidine Blue

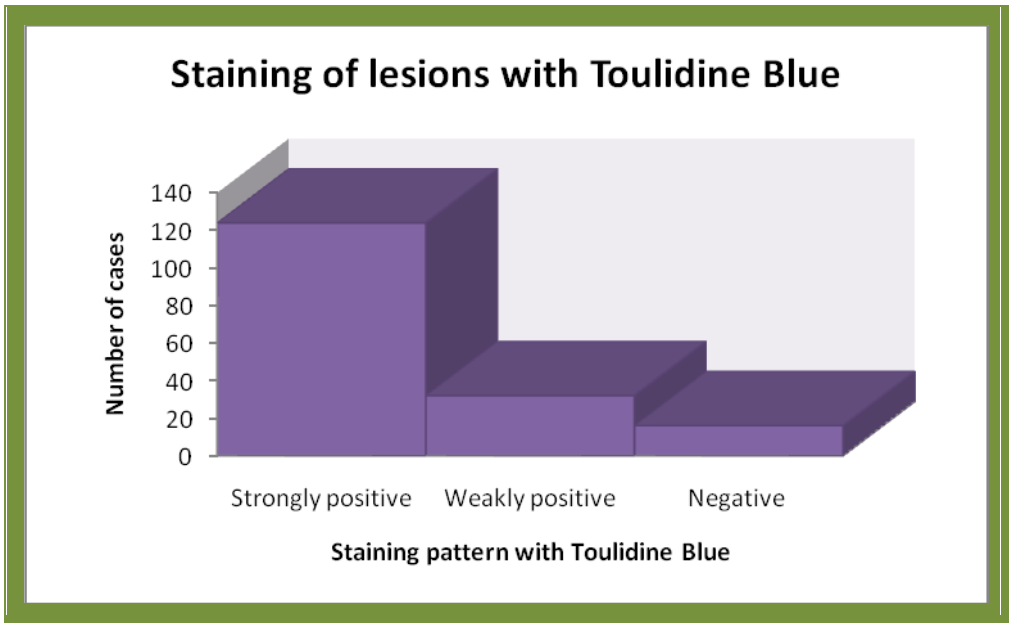


Figure 13
Brush Biopsy results

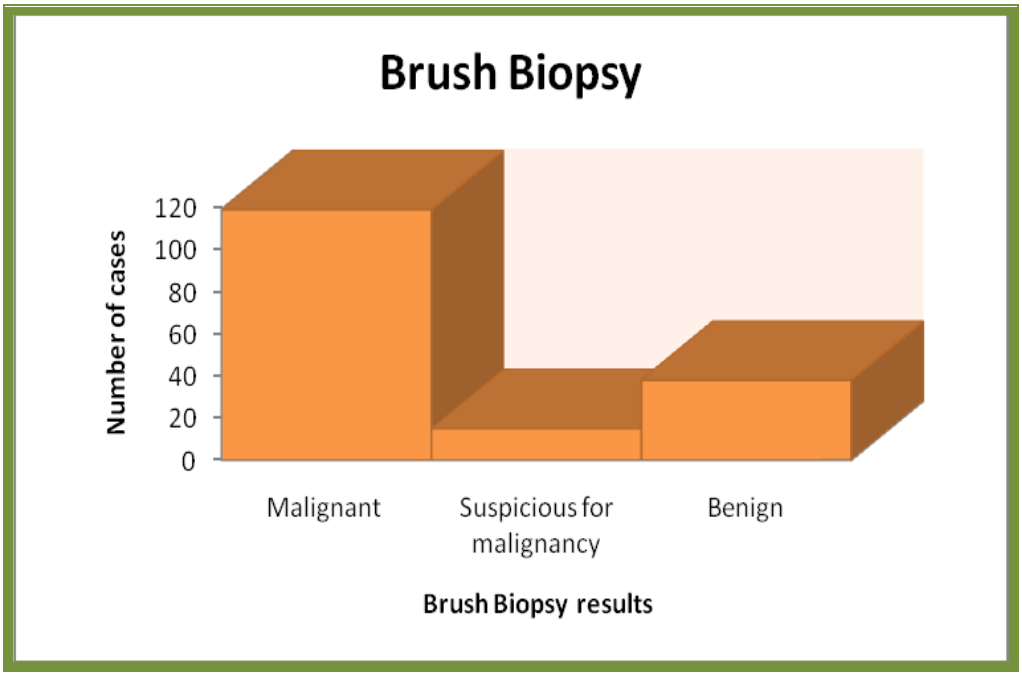


Figure 14
Histopathology of oral cancers

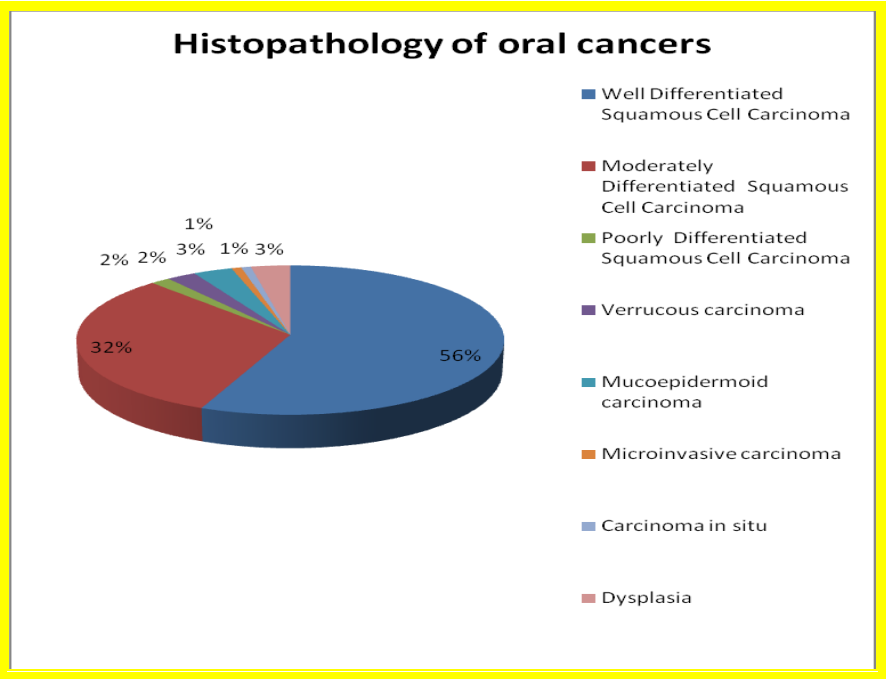


Figure 15
Histopathology of oral premalignant lesions:

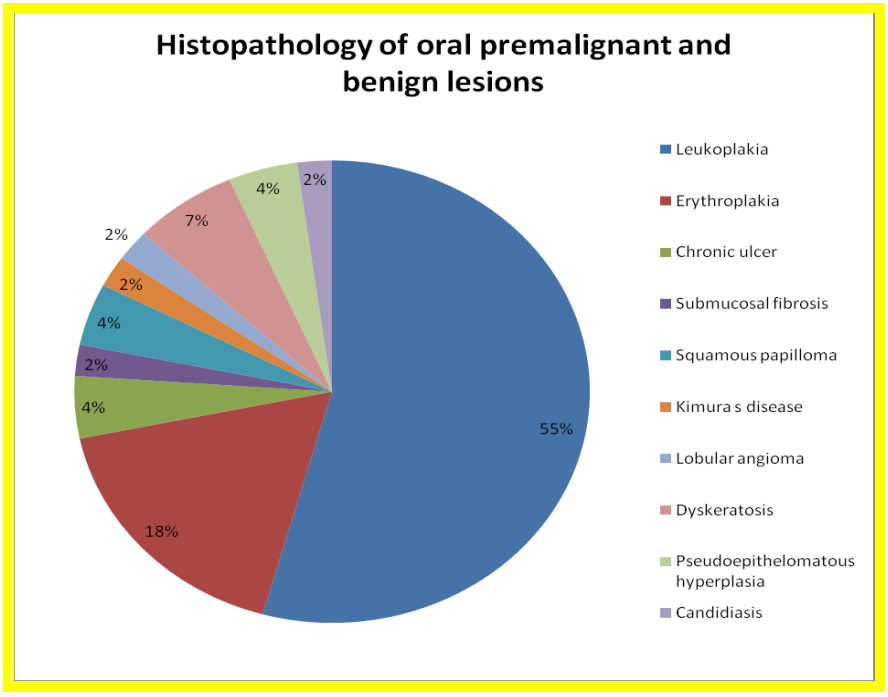


Figure 16
Toulidine blue showing strong positivity

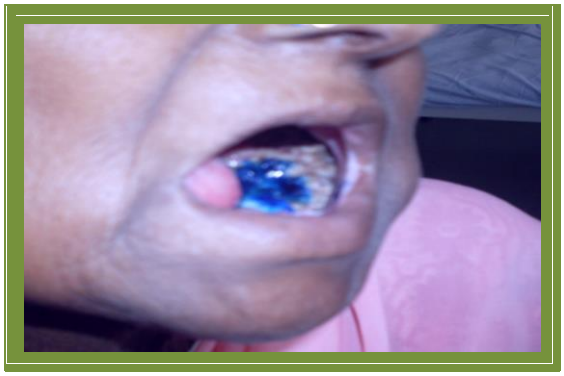


Figure 17

Toulidine blue showing weak positivity

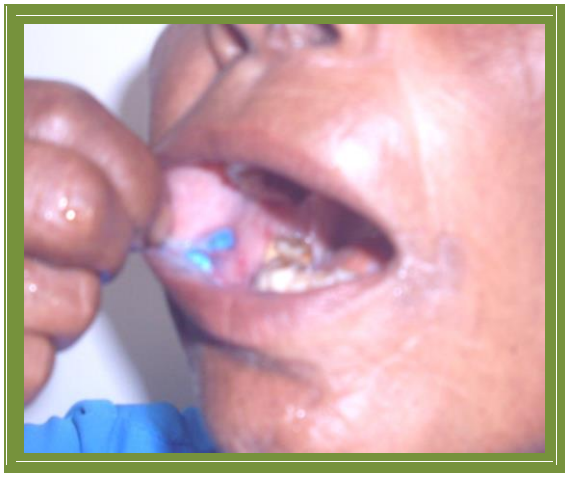


Figure 18
The negative staining of toulidine blue

Figure 19
Brush biopsy showing malignancy features

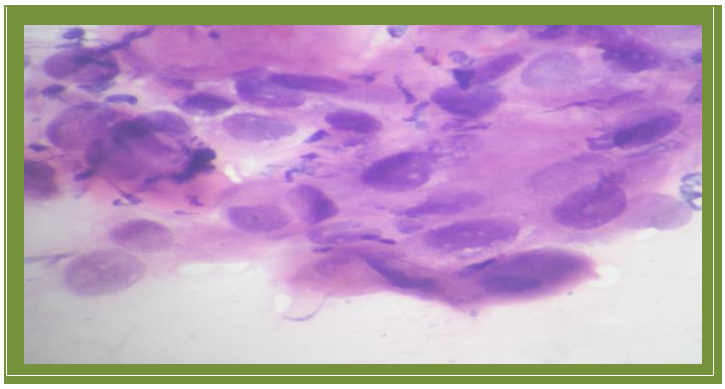


Figure 20
Brush biopsy showing suspicious for malignancy

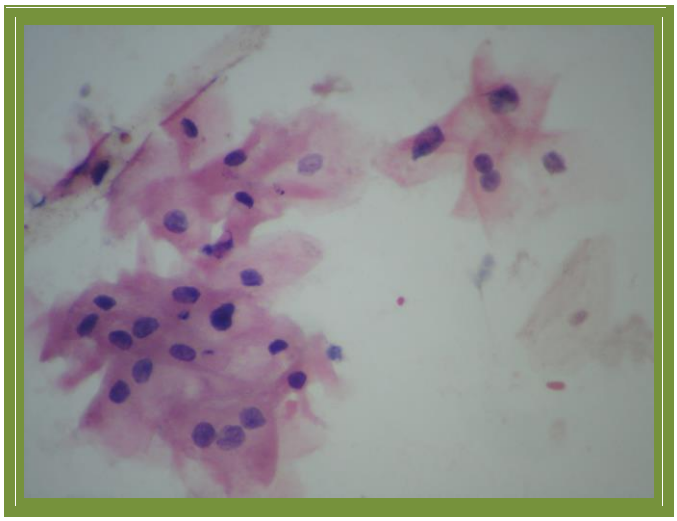


Figure 21
The brush biopsy showing benign squamous cells

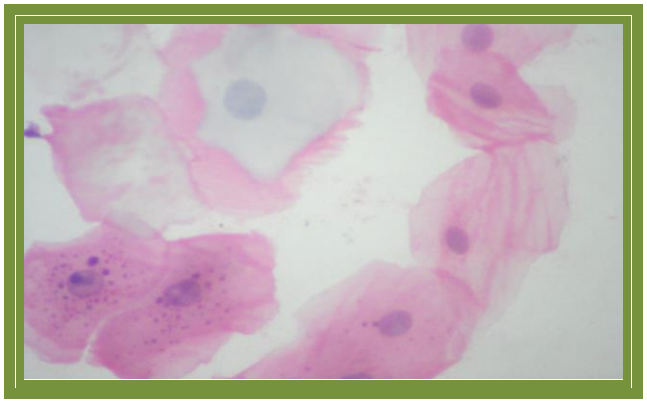


Figure 22
Case of mucoepidermoid carcinoma showing negative staining of toulidine blue

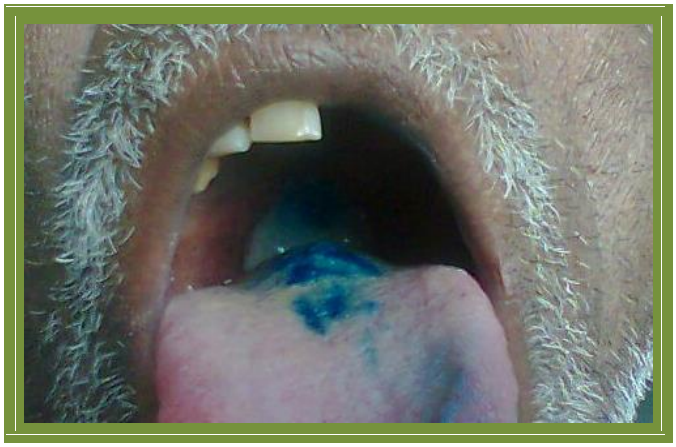


Figure 23
The brush biopsy of the above case showed mucinous material and few squamous cells

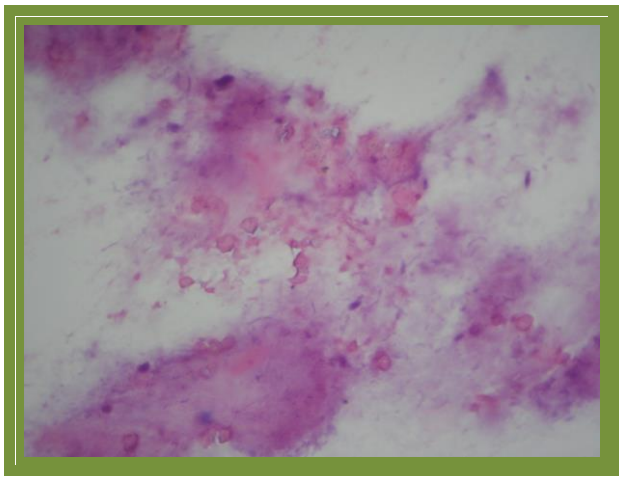
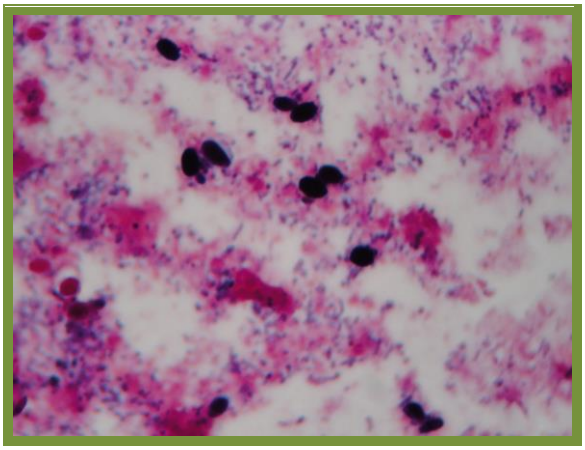


Figure 24
Case of oral candidiasis



Discussion

DISCUSSION

Oral cancer is very common in India constituting (30%) of cancer load [9]. A study conducted showed that about 20% of all cancer seen in ENT and oncology department were oral malignancies. Individuals having habit of smoking, alcohol consumption, areca nut chewing were reported to have 123 times the risk of developing cancer than people without such habits [9].

Clinical inspection alone is insufficient in identifying precursor lesions and early cancers. The difference in 5 year survival rate between early and late detection of oral squamous cell carcinoma is 80.55 % vs 18.3% making early detection of this disease a very important goal that all health workers should strive to achieve [23].

Epidemiological and clinical aspects :

Comparison of age groups and male:female (M:F) ratio in different studies are given in table 31

Table 31

Comparison of age groups and male:female (M:F) ratio

	Varshney et al [28]	Gupta et al [2]	Mehrotra et al [29]	Present study
Age group(years)	40-60	35-75	50-59	25-80
Mean	50	52	54	55
M:F ratio	4.56:1	3:1	3.27:1	1:2.8

Our study showed that oral cancers in this region were more common in female patients,in contrast to other studies mentioned above. This is probably because these studies were done in North India. Chewing tobacco/pan (quid) is fairly common practice among women in South India.

Among carcinogens, tobacco (84%) was the most common substance of abuse in one form or the other ,similar to earlier studies.

Lesion site:

The comparison of lesion site in various studies are given in table 32

Our study in comparison to other studies have shown that buccal mucosa and tongue are the predominant sites of oral cancers.

Table 32

The comparison of lesion site in various studies

	Gupta et al[2]	Mehrotra et al[29]	Present study
Maximum lesion site	Tongue and buccal mucosa	Tongue and buccal mucosa	Tongue and Buccal mucosa
%	63	57	75

The anterior two thirds of the tongue is commonly involved in India,while the posterior lateral border and ventral surfaces are frequently involved in the United States. These regional differences may be attributed to the extensive use of chewing tobacco in the Indian subcontinent compared to smoking in the West with the incidence being highest at mucosal sites with prolonged contact with carcinogens.

Mehrotra et al [29] detected 63 new cases of oral cavity per annum. Our centre detects 81 new cases of oral cavity per annum. Properly structured site specific data like this can augment National Cancer Registry Programme (NCRP) and is essential indicator for magnitude and pattern of cancer problem in India. [29]

Varsney et al [28] showed that maximum cases 52% with 15-20 years exposure to carcinogens had developed cancer. Our study showed maximum patients 68% who were exposed for a period of 21-30 years developed cancer.

In our study clinical diagnosis showed false negative rate of 6.34% and false positive rate of 21.73%, similar to earlier study who had false negativity of 4.8% and false positivity of 28.5%[23].

Most of our cases of carcinomas showed Well Differntiated Squamous Cell Carcinoma 41%, and among premalignant lesions leukoplakia were more common 15%, similar to other studies by Gupta et al [2] (56%) and Mehrotra et al [29] (67%) .

Adjunct techniques to detect malignant and premalignant oral lesions.

Supravital toulidine blue staining:

An easy economic technique is required for the purpose of mass screening. Supravital staining method has been adopted to aid in early diagnosis since years. Toulidine blue (supravital dye) was first used by Richart in 1963 in medicine for detecting cervical dysplasia and carcinoma in situ [9].

Toulidine blue (TB) is a cationic metachromatic vital dye that binds to sulphates, phosphates and carboxylates. Cells which are dysplastic or cancerous have leaky cell membrane ,allowing the dye to enter and bind to phosphate group of nucleic acids. Also malignant epithelial cells may contain intracellular canals that are wider than normal epithelium,which may facilitate penetration of the dye. Hence it is used as vital stain to highlight potentially malignant oral lesions, outline full extent of dysplastic epithelium or cancer when excision are planned. It also helps to follow up of patients with oral cancer. It is most useful in selecting the biopsy sample site [2,30].

In our study after screening of patients with Toulidine Blue application , the cases were categorized as strongly positive (figure 16) , weakly positive (figure 17) and negative (figure18). Among 124 lesions 116 carcinoma cases showed strong positivity. The other lesions which were false positives are described later (table 24). Among the weak positives all were premalignant and few cases of carcinoma in situ (1) microinvasive carcinoma (1) and dysplasia (4) suggesting that weak positivity is also a good indicator of premalignant/early malignant lesions. These cases need to be followed up as they may progress to cancers.

Sensitivity in the published data for TB ranged from 86% to 100%. In our study , invivo TB staining was highly sensitive and efficient in detecting malignant disease (92%).

Studies evaluating the application of toluidine blue in oral lesions are shown in table 33

Table 33

Studies evaluating the application of toluidine blue in oral lesions

Study	Sensitivity (%)	Specificity (%)
Niebel and Chomet [31]	100	100
Shed et al [6]	100	75
Myers [32]	100	100
Vahidy et al [33]	86	76
Reddy et al [34]	99	88
Mashberg [23]	96	95
Mashberg [25]	90	91
Silverman et al [24]	98	90
Epstein et al [35]	92	63
Onofre et al [3]	92	44
Warnakulasuriya and Johnson[36]	86	62
Martin et al [37]	100	-
Gupta et al [2]	97	86
Hegde et al [38]	97	62
Present study	92	82

Warnakulasuriya et al[36] found TB staining to be less usefull in detecting premalignant lesions. They found a false negative rate for oral epithelial dysplasia of 22%. Martin[37] and collegues found false negative staining rates for carcinoma in situ of 42% and 58%,respectively for moderate and severe dysplasia. We had 10 cases of false negatives of which were cases of mucoepidermoid carcinoma (4), microinvasive carcinoma (1), dysplasia (4), carcinoma in situ (1).

In aspect of specificity of TB staining, we obtained a value of 82.60% with a resulting false positive rate of 17.39%. the false positives were 8 cases leukoplakia (4) , erythroplakia (2), chronic ulcers (2). These false positives could be related to the retention of stain in inflamed and trauma areas. Other causative factors may include the irregular, papillary or digital surfaces of the lesions, which may cause the mechanical retention of dye , contamination of saliva and plaque, retention of dye material in papilla of the tongue or minor salivary gland ducts over mucosa[9].

For TB , false positive results are quite common in ulcerated ,inflammatory , or traumatic lesions as reported by previous workers.

TB appears to stain only 3-4 cell layers deep. Therefore ,early SCC that might be surfaced by intact epithelium, which are not exposed to environment, do not stain[9].

In our study, 2 patients with mucoepidermoid cancers (figure 22) presented as plaques, did not take the stain. It is important to understand that only those lesions which have some ulceration of mucosa will take the stain. Those which are completely mucosal covered will not. This Suggest that TB is not sensitive in detecting salivary gland tumors

Recently , Nagaraju et al[30] tried to assess sensitivity of TB with lugol s iodine,their sensitivity was 100%, specificity was 60%. Lugol s iodine when used with toulidine

blue helped in delineating inflammatory lesions. The malignant lesions without glycogen content failed to show lugol s iodine content .

Zhang et al[39],found that more than 6 fold elevation in cancer risk was observed for TB positive lesions with positive retention of dye present in 12 of 15 lesions,that later progressed to cancer. (p=0.0008).

Martin et al[37] demonstrated TB to be highly efficient in detecting invasive malignant disease with a sensitivity of 100%.

A recent prospective longitudinal study showed TB staining and allelic loss is predictive of malignant transformation, even in benign lesion or those with low grade dysplasia and is important for further study and use of TB[39].

A 2 week waiting period is required before staining, assuming that patients will return for follow up, will reduce false positive findings, secondary to inflammatory condition in a general population[23].

Exfoliative cytology :

Advances in the early detection of oral cancer are unfolding and analogous to those made in the advances in cervical carcinoma. In the early 1950s cervical cancer was the 2nd leading cause of cancer death. By late 1960s cervical cancer dropped to the 7th leading cause of cancer death, all because of widespread utilization of brush cytology, specifically the cervical papanicolau (PAP) smear. These changes have resulted in reduction of cervical cancer death by 74%.

Brush cytology has the potential to assist the diagnostic portion of the "screening gap" which currently challenges the early detection of many epithelial cancers. It is a noninvasive technique,based upon the fact that dysplasia and cancer cells tend to "slough off" or exfoliate preferentially and can easily be collected from surface of the lesion [4].

The oral Brush Biopsy was introduced to dental profession in 1999 which uses special brush to scrape all 3 layers of lesion, basal,intermediate and superficial layers. It does not require topical anesthesia and causes minimal bleeding and pain.

Full thickness sampling indicated by pinpoint bleeding during procedure, is essential for evaluation of collected cells to yield representative findings. Many dysplastic cells first develop in basal layer may be lost as the cells mature and keratin are produced[10].

In our study all 172 cases were screened with BB and the cytological smears were categorized as malignant (figure19), suspicious for malignancy (figure 20) and benign (figure 21). Of 119 cytologically malignant cases 115 truly matched with histopathology report as cancers, suggesting that BB is highly sensitive in oral cancers. The other lesions which were false positives (table 25) are described later.

The cases which were suspected of malignancy on cytology were diagnosed as verrucous carcinoma 1, mucoepidermoid carcinoma 2, microinvasive carcinoma 1, carcinoma in situ 1, dysplasia 4, erythroplakia 3, pseudoepithelomatous hyperplasia 2, chronic ulcer 1 on histopathology. Hence cases of suspicious for malignancy should be treated in light of malignancy and should be confirmed with histopathological examination.

2 cases of mucoepidermoid carcinoma on cytology showed only mucous and few squamous cells as shown in (figure 23). One case presented as leukoplakia but on cytology showed budding forms of candida (figure 24)

The true BB negatives were truly benign on histopathology suggesting that BB is specific for benign lesions.

Studies evaluating the utility of brush biopsy in oral lesions are shown in table 34

Table 34

Studies evaluating the utility of brush biopsy in oral lesions

Study	Sensitivity (%)	Specificity (%)
Mehrotra et al[40]	76	93
Errickson[41]	71	100
Gupta et al[2]	89	92
present study	91	91

Similar to other studies we had sensitivity 91% and specificity 91% of BB.

Potter et al[42] found 4 BB false negatives of a total 115 cases analysed. Although the number of false positive cases is small in his study it is important to emphasize that mean delay time in diagnosing a cancer in these cases was 117.25 days.

The false negative rate of BB can exceed 30% as the cytology instruments do not sample deepest layers of oral lesions.

Our false negative rate for BB was 8.7%, among them were cases of mucoepidermoid carcinoma (4),verrucous carcinoma (1),dysplasia (4),microinvasive carcinoma (1),and carcinoma in situ (1).

Our false positivity for BB was 8.6%,among them were cases of dyskeratosis (2),and chronic ulcer (2).

Problems with BB are mainly due to the existence of false negative obtained as a result of a non representative sample as well as the subjective cytological examination.

Mehrotra et al[40] studied 79 patients with adequate transepithelial BB. Their sensitivity was 76.8% ($p<0.5$) and specificity was 93.3%.They had 4 false negatives which turned out to be dysplasia/malignancy on histopathology.

The biggest pitfall with BB is risk of false negative results if sample is too superficial. Dysplastic lesions might show normal cytology results in the upper layers of oral mucosa, therefore a sufficient number of cells must be removed to reach the deeper cell layer of the lesion. It has been suggested that a sufficient sample should cause pinpoint bleeding at the site of lesion.

False negative test results using BB have been reported. So a negative cytology result must be interpreted in the light of clinical pretest probability of cancer. Highly suspicious lesions with negative BB results should be repeated or the patient should be advised for an excisional biopsy[43].

Svirsky et al[44] studied 243 patients with abnormal BB and showed 38% positive predictive value, suggesting that these patients have strong positive predictive value for dysplasia or cancer.

Combined TB and BB evaluation for malignant lesions of our study and study by Gupta et al[2] are shown in table 35

Table 35

Combined TB and BB evaluation for malignant lesions of our study and study by Gupta et al[2]

	Gupta et al[2]	Present study
Sensitivity (%)	97	93
Specificity (%)	90	95
Positive predictive value (%)	91	99
Negative predictive value (%)	96	84

Combined TB and BB evaluation for premalignant lesions of our study and study by Gupta et al[2] are shown in table 36

Table 36

Combined TB and BB evaluation for premalignant lesions of our study and study by Gupta et al[2]

	Gupta et al[2]	Our study
Sensitivity (%)	93	88
Specificity (%)	85	90
Positive predictive value (%)	86	96
Negative predictive value (%)	92	69

Similar to study by Gupta et al[2], combined evaluation of toluidine blue staining and oral brush biopsy showed an increase in sensitivity in premalignant cases, but in malignant cases , no additional advantage was obtained.

Rammerbach et al[45] studied 1328 exfoliative smears of which 332 lesions were compared with histology. Additionally , nuclear DNA content was measured after Feulgan restaining using a TV image analysis system. The sensitivity of cytologic diagnosis in addition to DNA image cytometry on oral smears for detection of cancer cells was 98%, specificity 100%, positive predictive value 100% and negative predictive value 98%. However, in our study DNA image cytometry was not done . Highly sophisticated diagnostic technique like cytomorphometry, DNAcytometry and molecular analysis have become the recent trend.

Umudum et al[46] have studied presence of Human Papilloma Virus DNA by insitu hybridization (ISH) in metastatic lesions from Squamous Cell Carcinoma ,using alcohol fixed, archival cytopathological material. How ever this was in oropharynx.

Ki 67 has been studied in oral cytological smears using Immuno Histo Chemistry to evaluate the nature of lesion and response to treatment[47].

Though molecular markers was not used in our study, further study including epigenetic alterations(hypermethylation of promoter regions) and genomic instability such as loss of heterozygosity and micro satellite instability can help in knowing the pattern of carcinogenesis.

Further studies using Polymerase Chain Reaction is on way to detect tumoral DNA for monitoring recurrences in patients. Loss of heterozygosity (LOH) and other molecular changes indicating oral carcinogenesis have been identified in exfoliated cells.

Huang et al[48] have used PCR technique to amplify DNA from exfoliated cytology sample from oral cancer for analysis of restriction fragment length polymorphism (RFLP). They found that 66% of tumors studied showed LOH at one position in the p53 sequence while 55% showed LOH at some other location.

The current gold standard of diagnosis of premalignant lesions is histopathology. However, intraobserver, interobsever findings vary among pathologist in the diagnosis of mild to moderate dysplasia that comprise the largest proportion of premalignant disease and in determining early stage invasion of Carcinoma In Situ or Squamous Cell Carcinoma.

In conclusion , this study has found that the combined use of TB staining of oral lesions followed by BB cytological study has a high degree of sensitivity in detecting malignant and premalignant lesions. This is an easy, non invasive and inexpensive procedure for screening any oral lesions. This can then be followed up, when required by Histopathological confirmation.

CONCLUSION

*This study suggests that early detection of oral cancer is possible even at precancerous stage by using noninvasive , painless outpatient procedure of combined invivo supravital Toluidine blue staining and Brush biopsy.

*Toluidine blue showed a few false positive results, especially in inflammatory lesions.

*Combined evaluation of toluidine blue and brush biopsy has helped to increase the sensitivity and specificity in detecting premalignant lesions and also to minimize false negatives.

*This combined technique is an ideal screening tool and can act as a potential practical procedure in a resource challenged setting like rural India which bears the brunt of oral cancer burden.

SUMMARY

The findings in our study can be summarized as follows

1. A total of 172 clinically suspected of premalignant and malignant oral lesions were included in our study.

2. Maximum number of cases were in the age group of more than 40 years.

3. Our study showed female preponderance of cases M:F ratio 1:2.8.

4. Maximum cases had a habit of areca nut/tobacco chewing 84%

5. Most of the lesions were seen in buccal mucosa 62%

6. Clinically most of the carcinoma cases had pain ,halitosis and lymphnode enlargement.

7. Histopathology diagnosed 73% carcinomas and 26% premalignant and benign lesions among total 172 cases.

8. Sensitivity and specificity of TB in malignant lesions were 92% and 82% respectively.

9. Sensitivity and specificity of BB in malignant lesions were 91% and 91% respectively.

10. Sensitivity and specificity of TB in premalignant lesions were 61% and 80% respectively.

11. Sensitivity and specificity of BB in premalignant lesions were 83% and 90% respectively.

12. Sensitivity and specificity of combined TB and BB in malignant lesions were 93% and 95% respectively.

13. Sensitivity and specificity of combined TB and BB in premalignant lesions were 88% and 90% respectively.

14.Combined evaluation of toluidine blue staining and oral brush biopsy showed an increase in sensitivity in premalignant cases, but in malignant cases , no additional advantage was obtained.

Bibliography

Bibliography

1. Siddiqui I,Farooq U, Rafi T . Role of Toluidine Blue in Early Detection of Oral.Pakistan. J of Med Sci 2006;22(2):184-187
2. Gupta A,Singh M,Ibrahim R,Mehrota R. Utility of toluidine blue staining and brush biopsy in precancerous and cancerous oral lesions .Acta Cytol 2007;51(5):788-94
3. Onofre MA,Sposto MR, Navarro CM, Motta ME, Turatti E,Almeida RT. Reliability of toluidine blue application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001 May;91(5):535-403
4. Gandolfo S,Pentero M,Broccoletti R,Pagano M,Carrozo M,Scully C. Toluidine blue uptake in potentially malignant oral lesions in vivo: Clinical and histological assessment. Oral Oncol 2006 ;42(1):89-95
5. Epstein JB, Feldman R, Dolar RJ.The utility of tolonium chloride rinse in the diagnosis of recurrent or second primary cancers in patients with prior upper aerodigestive tract cancer.Head and Neck 2003 ;25(11):911-211
6. Shedd DP,Hukill PB,Bahn S. In vivo staining properties of oral cancer.Am J Surg 1965;110:631-634
7. Strong MS,Vaughan CW,Inc Za JS. Toluidine blue in the management of carcinoma of oral cavity. Arch Otolaryngol 1968;87:527-531
8. Brad W. Neville and Terry A. Oral Cancer and Precancerous Lesions . Ca Cancer J Clin 2002;52;195-215

9. Chan Y,Lin J,Wu C,Lui M.Application of In Vivo Stain of Methylene Blue as a Diagnostic Aid in the Early Detection and Screening of Oral Squamous Cell Carcinoma and Precancer Lesions.J Chin Med Assos 2007;70:497-503
10. Mehrotra R, Gupta A, Mamta S, Ibrahim R .Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions .Mol Cancer. 2006; 5: 11.
11. Sussan.Oral cavity.In : Gray 's Anatomy 14th edition. Churchill Livingstone: Elsevier; 2008:581-590
12. Petti S, Scully C .Oral cancer: the association between nation-based alcohol-drinking profiles and oral cancer mortality.Oral Oncol. 2005; 41(8):828-34
13. Joanna M ,Zakrzewska.Oral cancer.Brit Med J. 1999; 318: 1051–1054.
14. Park K. Oral cancers. In: Preventive and Social Medicine 19th edition. Jabalpur: Banarsidas Bhanot;2007:323
15. Farland M, Abaza N, Elmofthy. In: Anderson s Patholgy 10th edn . Missouri: Elsevier ; 1996:1583-1589.
16. Nigel K.In:Lever s Histopathology of the Skin 9th edn. Philadelphia:Lippincott Williams and Wilkins;2006: 824-827
17. Rosai J . In:Rosai and Ackerman s Surgical Pathology 9th edn . New York: Elseiver; 2004:252-259
18. Silverberg S , Delellis R , Frable W.In:Principles and practice of Surgical Pathology and Cytopathology 3rd edn. New York : Churchill Livingstone;1997:1428-1441
19. Kenneth D,Richard J. In : Sternberg s Diagnostic Pathology 4th edn. Philadelphia: Lippincott Williams and Wilkins; 2003.

20. Leopold G.K.In: Koss Diagnostic Cytology 5th edn . Newyork :Lippincott Williams and Wilkins ;2006.
21. Gillenwater A,Jacob R,Ganeshappa R. Noninvasive Diagnosis of Oral Neoplasia Based on Fluorescence Spectroscopy and Native Tissue Autofluorescence. Arch Otolaryngol Head Neck Surg 1998;124:1251-1258.
22. Lauren L, Joel B, Epstein J, and Ross K. Adjunctive Techniques for Oral Cancer Examination and Lesion Diagnosis.A Systematic Review of the Literature. J Am Dent Assoc 2008;139: 896-905
23. Mashberg A .Diagnosis of early oral and oropharyngeal squamous carcinoma.Oral Oncol 2000;36:253-255
24. Silverman S,Migliorati C.Toluidine blue staining and early detection of oral precancerous and malignant lesions.Dent J 1992;78:15-16
25. Arthur Mashberg.Reevaluation of toluidine application as a diagnostic adjunct in the detection of asymptomatic oral squamous cell carcinoma . Cancer 1980;46:758-763
26. Tyler J. Potter, D, Summerlin D, John H. Campbell, *D.*Oral Malignancies Associated With Negative Transepithelial Brush Biopsy. J Oral Maxillofac Surg 2003;61:674-677
27. Mehrotra R, Singh M, Shruti P, Mamta S. The use of an oral brush biopsy without computer-assisted analysis in the evaluation of oral lesions: a study of 94 patients.Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:246-53
28. Varshney PK, Agarwal N, Bariar LM. Tobacco and alcohol consumption in relation to oral cancer .Indian journal of otolaryngology and head and neck surgery 2003; 55:25-28

29. Mehrotra R, Singh M, Kumar D, Pandey AN, Gupta RK, Sinha US. Age specific incidence rate and pathological spectrum of oral cancer in Allahabad. Indian J Med Sci 2003;57:400-4
30. Nagaraju K, Prasad S, Ashok L.Diagnostic efficiency of toulidine blue with Lugol' s iodine in oral premalignant and malignant lesions.Indian Journal of Dental Research 2010;21:218-223
31. Niebel HH, Chomet B. In vivo staining test for delineation of oral intraepithelial neoplastic change: preliminary report. J Am Dent Assoc 1964;68:801-6.
32. Myers EN. The Toluidine Blue Test in Lesions of the Oral Cavity. Cancer J Clin 1970;20;134-139
33. Vahidy NA, Zaidi SHM, Jafarey NA. Toluidine blue test for detection of carcinoma of the oral cavity: an evaluation. J Surg Oncol 1972;4:434-8.
34. Reddy CRRM, Ramulu C, Sundareshwar B, Raju MVS, Gopal R, Sarma R. Toluidine blue staining of oral cancer and precancerous lesions. Indian J Med Res 1973;61:1161-4.
35. Epstein JB, Scully C, Spinelli JJ. Toluidine blue and lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. J Oral Pathol Med 1992;21:160-3.
36. Warnakulasuriya KAAS, Johnson NW. Sensitivity and specificity of OraScan toluidine blue mouthrinse in the detection of oral cancer and precancer. J Oral Pathol Med 1996;25: 97-103.

37. Martin I C, Kerawala C J, Reed M. The application of toulidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. Oral Surg Oral Med Oral Pathl Oral Radiol Endod 1998;85:444-6.
38. Hegde MC, Panduranga M. Kamath, Shreedharan S, Dannana NK, Raju RM. Supravital Staining: It's role in detecting early malignancies . Indian Journal of Otolaryngology and Head and Neck Surgery 2006;58:31-34
39. Zhang L, Williams M, Catherine F, Laronde D,Joel B, Epstein, Durham S, Nakamura H et al .Toluidine Blue Staining Identifies High-Risk Primary Oral Premalignant Lesions with Poor Outcome. Cancer Res 2005; 65(17):8017-8021
40. Mehrotra R, Singh MK, Pandya S and Singh M. The use of an oral brush biopsy without computer-assisted analysis in the evaluation of oral lesions: A study of 94 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:246-53
41. Eriksson AT , Corcuera MM , Trapero JC, Sánchez JC , Martínez AB. Analysis of new diagnostic methods in suspicious lesions of the oral mucosa.Med Oral Patol Oral Cir Bucal 2009;14 (5):210-6.
42. Potter TJ, Summerlin DJ, and John H. Campbell. Oral Malignancies Associated With Negative Transepithelial Brush Biopsy J Oral Maxillofac Surg 2003;61:674-677
43. Naugler C. Brush biopsy sampling of oral lesions. Can Fam Physician 2008 ; 54(2): 194
44. Svirksy JA,Burns JC,Page DG,Abbey LM. Computer assisted analysis of the oral brush biopsy . Compend Contin Educ Dent 2001;22:99-102.

45. Remmerbach TW, Mathes SN, Weidenbach H,Hemprich A,Bocking A.Non invasive brush biopsy as an innovative tool for early detection of oral carcinomas. Mund Keifer Gesichtschir 2004;8:229-236.
46. Umudum H, Rezanko T, Dag F, Dogruluk T. Human papillomavirus genome detection by in situ hybridization in fine-needle aspirates of metastatic lesions from head and neck squamous cell carcinomas. Cancer Cytopatholgy 2005;105:171-176
47. Sharma P, Kumar N, Bahadur AK, Mandal AK. Ki-67 expression in cytologic scrapes from oral squamous cell carcinoma before and after 24 Gray radiotherapy- a study on 43 patients. Med Oral Patol Oral Cir Bucal. 2005;1:15–17
48. Huang MF, Chang YC, Liao PS, Huang TH, Tsay CH, Chou MY. Loss of heterozygosity of p53 gene of oral cancer detected by exfoliative cytology. Oral Oncol 1999;35:296–301.

Annexures

PROFORMA : (ANNEXURE A)

Serial number : Hospital number:

Name : Age: Sex:

History :

Pain/ white lesion/ ulcer / fibrosis / trismus / halitosis /lymph node enlargement /weight loss/ others

Duration of complaints of the lesion:

Past history:

Smoking/ alcohol consumption/ pan,tobacco chewing

Duration of exposure to carcinogens:

On examination :

Lesion site:

Buccal mucosa/ floor of the mouth / hard palate / lip / tongue anterior 2/3rd / upper alveolus/ lower alveolus/ retro molar trigone

Size of the lesion :

2cm/ 2-4cm/ > 4cm

Lesion type :

Plaque/ ulcerative/ proliferative/ ulceroproliferative

Clinical diagnosis:

Application of 1% toulidine blue stain result:

Strongly positive/ weakly positive/ negative

Brush biopsy result:

Adequacy

Atypical cells : present/absent

N/C ratio: normal/increased

Nuclear chromatin: normal/ vesicular/ hyperchromatic

Nucleoli : present / absent

Cytological diagnosis:

Benign/ suspicious for malignancy/ malignant

Histopathology:

Biopsy number:

Histopathology diagnosis:

Key to Master chart (Annexure B)

Sl no:serial number **Bx no**:biopsy number

Age in years

Sex : F:female**,** M:male

Presenting complaints:

Pain : yes (Y), no (N)

Plaque : yes (Y), no (N)

Ulcer: yes (Y), no (N)

Fibrosis: yes (Y), no (N)

Trismus : yes (Y), no (N)

Halitosis : yes (Y), no (N)

Weight loss : yes (Y), no (N)

Others: yes (Y), no (N)

Duration of lesion in months

P.History:past history

Smoking: yes (Y), no (N)

Alcohol : yes (Y), no (N)

Pan/beetel nut: yes (Y), no (N)

Duration of carcinogen in years

Site of the lesion:

Buccal mucosa

Floor of the mouth

Hard palate

Lip

Tongue anterior 2/3rd

Upper alveolus

Lower alveolus

RMT: retro molar trigone

Lesion size in cm

<2cm, 2-4cm, >4cm

Lesion type:

Plaque /Ulcerative/Ulceroproliferative

Clinical diagnosis:

Leukoplakia

Erythroplakia

Ca:carcinoma

TB:toulidine blue

s. positive:strong positive

w. positive:weak positive

negative

Brush biopsy

Adequacy**:** yes (Y), no (N)

Benign squamous cells : present (+), absent (-)

Atypical cells : present (+), absent (-)

N/C : nuclear/ cytoplasmic ratio

Increased /normal

Chromatin : normal,vesicular,hyperchromatic

Nucleoli : present (+), absent (-)

NM: nuclear membrane: regular/ irregular

Mitosis : yes (Y), no (N)

Cyto diag:cytology diagnosis

Benign/malignant/suspicious for malignnacy

Histo diagnosis:histology diagnosis

Malignant lesions:

WDSCC: Well Differentiated Squamous Cell Carcinoma

MDSCC: Moderately Differentiated Squamous Cell Carcinoma

PDSCC: Poorly Differentiated Squamous Cell Carcinoma

Verrucous carcinoma

Mucoepidermoid carcinoma

Microinvasive carcinoma

Carcinoma in situ

Dysplasia

Benign lesions:

Leukoplakia

Erythoplakia

Chronic ulcer

Submucosal fibrosis

Kimura s disease

Lobular angioma

Dyskeratosis

Pseudoepithelomatous hyperplasia

candidiasis

MASTER CHART

				presenting complaints										P .HISTORY											brush biopsy					
Sl No	Biopsy Number	Age in year	Sex	Pain	plaque	Ulcer	fibrosis	Trismus	Halitosis	Lymphnode	Wt loss	Others	Duration in months	Smoking	Alcohol	pan/beetel nut	Duration of pan yrs	Site of the lesion	Lesion size in cm	Lesion type	Clinical Diagnosis	TB	Adequacy	Benign squamous cells	Atpical cells	N/C	Chromatin	Nucleoli	NM	Mitosis	Cyto diagnosis	Histo diagnosis
1	859/08	60	F	Y	N	Y	N	N	Y	Y	Y	N	6	N	N	Y	30	buccal mucosa	<2cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
2	918/08	50	F	Y	N	Y	N	N	Y	Y	N	N	3	N	N	Y	28	hard palate	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	hyperchromatic	+	irregular	N	malignant	PDSCC
3	990/08	40	F	N	Y	N	N	N	N	N	N	N	1	N	N	N	12	upper alveolus	<2cm	plaque	leukoplakia	s.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
4	1025/08	45	F	Y	N	Y	N	N	Y	Y	Y	Y	18	N	N	Y	20	buccal mucosa	2-4cm	ulcerative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	MDSCC
5	1118/08	48	F	N	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	30	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
6	1164/08	35	M	Y	Y	Y	N	N	Y	Y	Y	N	2	Y	Y	Y	18	buccal mucosa	<2cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
7	1184/08	50	F	Y	Y	N	N	N	N	N	N	N	5	Y	N	Y	25	buccal mucosa	<2cm	plaque	erythroplaki	w.positive	Y	+	-	normal	normal	-	regular	N	benign	erythroplakia
8	1232/08	35	F	Y	N	Y	N	N	Y	Y	Y	N	5	N	N	N	20	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	MDSCC
9	1246/08	55	F	N	N	Y	N	N	Y	Y	Y	N	4	N	Y	Y	25	upper alveolus	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
10	1272/08	80	F	N	Y	N	N	N	N	N	N	N	1	N	N	Y	15	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
11	1295/08	55	F	Y	N	Y	N	N	Y	Y	Y	Y	6	N	N	N	18	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	regular	N	benign	WDSCC
12	1300/08	50	F	N	N	Y	Y	N	Y	Y	Y	N	3	N	N	Y	16	lower alveolus	2-4cm	ulceroproliferative	ca	negative	Y	+	-	increased	vesicular	+	regular	N	benign	squamous papilloama
13	1315/08	48	F	Y	N	Y	Y	N	Y	Y	Y	N	6	N	N	Y	35	buccal mucosa	>4cm	ulcerative	ca	s.positive	Y	-	+	increased	hyperchromatic	-	irregular	N	malignant	WDSCC
14	1324/08	39	F	N	N	Y	N	N	Y	Y	Y	Y	3	N	Y	Y	30	buccal mucosa	>4cm	ulceroproliferative	ca	negative	Y	+	-	normal	normal	-	regular	N	benign	pseudoepihelomatous hyperplasia
15	1330/08	60	F	Y	N	Y	N	N	Y	N	Y	N	5	N	N	N	20	upper alveolus	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
16	1415/08	70	F	N	Y	N	N	N	N	N	N	N	2	N	N	Y	40	buccal mucosa	<2cm	plaque	leukoplakia	s.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
17	1658/08	42	M	N	N	Y	N	N	N	N	N	N	3	Y	Y	N	22	buccal mucosa	<2cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	MDSCC
18	1771/08	60	M	N	N	Y	N	N	Y	Y	Y	N	6	Y	Y	Y	40	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
19	1881/08	63	M	Y	N	Y	Y	N	Y	Y	Y	N	8	Y	N	Y	45	tongue ant2/3rd	<2cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
20	1890/08	30	M	N	N	Y	Y	N	Y	Y	Y	Y	12	Y	N	N	13	floor of mouth	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	MDSCC
21	16/09	73	M	Y	N	Y	N	N	Y	Y	Y	N	4	Y	Y	Y	50	hard palate	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	-	irregular	Y	malignant	WDSCC
22	20/09	32	M	Y	N	Y	Y	N	Y	Y	N	N	5	N	N	Y	15	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
23	21/09	48	F	N	N	Y	N	N	Y	Y	Y	N	3	Y	N	Y	24	upper alveolus	>4cm	ulceroproliferative	ca	negative	Y	+	-	increased	vesicular	+	regular	N	s.malignar	pseudoepihelomatous hyperplasia
24	29/09	70	F	Y	Y	Y	Y	N	Y	Y	Y	N	6	Y	N	N	45	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	-	normal	normal	-	regular	N	benign	verrucous ca
25	30/09	65	F	Y	N	Y	N	Y	Y	Y	Y	N	8	N	N	Y	36	upper alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
26	33/09	60	M	N	Y	N	Y	N	N	N	N	N	2	Y	N	Y	38	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
27	59/09	63	M	Y	N	Y	N	N	Y	N	N	N	5	Y	Y	N	40	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	negative	Y	+	-	increased	vesicular	+	irregular	N	benign	squamous papilloama
28	92/09	32	M	Y	N	Y	N	N	Y	Y	Y	N	3	Y	Y	Y	15	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	Y	malignant	PDSCC
29	115/09	68	F	Y	Y	N	N	N	N	N	N	N	1	N	N	Y	20	buccal mucosa	<2cm	plaque	erythroplaki	w.positive	Y	+	-	normal	normal	-	regular	N	benign	erythroplakia
30	130/09	45	M	Y	N	N	N	N	Y	Y	Y	Y	3	Y	Y	N	20	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
31	164/09	50	M	Y	N	Y	Y	N	Y	Y	Y	N	3	N	Y	Y	10	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
32	186/09	73	M	N	N	Y	N	N	N	Y	N	N	1	Y	N	Y	10	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC

33	228/09	45	M	Y	N	Y	N	N	Y	Y	Y	N	4	Y	N	N	20	tongue base	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	-	irregular	N	malignant	MDSCC
34	297/09	65	F	N	N	Y	Y	N	Y	Y	Y	N	2	N	N	Y	30	buccal mucosa	>4cm	ulceroproliferative	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	candidiasis
35	379/09	45	F	Y	N	Y	Y	N	Y	Y	Y	Y	1	Y	N	Y	15	RMT	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
36	391/09	45	F	Y	N	Y	N	N	Y	Y	Y	N	6	N	Y	Y	24	tongue ant2/3rd	>4cm	ulceroproliferative	ca	negative	Y	+	-	normal	normal	-	regular	N	benign	kimuras disease
37	398/09	70	M	N	N	Y	N	Y	Y	Y	Y	N	3	Y	Y	N	30	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	-	normal	normal	-	regular	Y	malignant	WDSCC
38	431/09	75	F	Y	N	Y	N	N	Y	Y	Y	N	20	Y	N	Y	40	buccal mucosa	2-4cm	ulceroproliferative	ca	negative	Y	+	-	normal	normal	-	regular	N	benign	lobular angioma
39	432/09	60	F	Y	N	Y	N	N	Y	Y	N	Y	5	N	N	Y	34	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
40	500/09	18	M	N	Y	N	N	N	N	N	N	N	3	N	N	N	0	hard palate	<2cm	plaque	ca	negative	Y	+	-	normal	normal	-	regular	N	s.malignar	mucoepidermoid ca
41	557/09	70	M	Y	N	Y	Y	N	Y	N	Y	N	4	Y	Y	Y	20	RMT	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
42	637/09	45	F	Y	N	Y	N	N	Y	Y	Y	Y	3	N	N	N	29	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
43	655/09	60	M	N	N	Y	N	N	Y	Y	N	N	2	Y	Y	Y	15	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
44	657/09	46	M	Y	Y	N	N	N	N	N	N	N	3	Y	Y	Y	18	tongue base	<2cm	plaque	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	s.malignar	dysplasia
45	669/09	75	F	Y	N	Y	N	N	Y	Y	Y	N	8	N	N	N	28	lower alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
46	693/09	40	F	Y	N	Y	Y	N	Y	Y	Y	N	3	N	N	Y	23	upper alveolus	>4cm	ulceroproliferative	ca	w.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	dyskeratosis
47	728/09	60	F	Y	N	Y	N	Y	Y	Y	Y	Y	5	N	N	Y	25	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
48	763/09	50	F	N	N	Y	N	N	Y	Y	N	N	24	Y	N	N	26	RMT	>4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
49	793/09	60	F	Y	N	Y	N	N	Y	Y	Y	N	2	N	N	Y	37	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	normal	-	irregular	Y	malignant	MDSCC
50	831/09	65	F	Y	N	Y	N	Y	Y	Y	N	Y	4	N	N	Y	20	lower lip	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	normal	+	irregular	N	malignant	WDSCC
51	849/09	70	F	Y	N	Y	Y	N	Y	Y	Y	N	3	N	N	N	25	lower lip	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	normal	+	irregular	N	malignant	MDSCC
52	857/09	45	F	Y	Y	N	N	N	N	N	N	N	2	Y	N	Y	23	RMT	2-4cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
53	858/09	35	F	N	Y	N	N	N	N	N	N	N	2	N	N	Y	8	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	dyskeratosis
54	880/09	55	F	Y	N	Y	N	N	Y	Y	Y	N	5	Y	N	N	20	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	normal	vesicular	+	irregular	Y	malignant	WDSCC
55	933/09	50	F	Y	N	Y	N	N	Y	N	N	N	1	N	N	Y	23	buccal mucosa	2-4cm	ulceroproliferative	ca	w.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	dyskeratosis
56	1002/09	55	F	N	Y	N	N	N	N	N	N	N	2	N	N	Y	14	buccal mucosa	<2cm	plaque	leukoplakia	s.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
57	1086/09	80	F	Y	N	Y	N	N	N	N	N	N	3	Y	N	N	45	hard palate	<2cm	ulceroproliferative	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
58	1110/09	75	F	Y	N	Y	Y	N	Y	N	Y	N	4	N	N	Y	30	buccal mucosa	2-4cm	ulceroproliferative	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
59	1111/09	60	F	N	N	Y	N	N	N	Y	Y	yy	8	N	Y	Y	22	buccal mucosa	>4cm	ulceroproliferative	submucosal	negative	Y	+	-	normal	normal	-	regular	N	benign	submucosal fibrosis
60	1120/09	71	M	Y	N	Y	N	N	Y	Y	Y	N	6	Y	Y	Y	46	tongue base	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
61	1167/09	50	F	Y	N	Y	N	N	Y	Y	Y	N	4	N	N	N	26	buccal mucosa	2-4cm	ulceroproliferative	leukoplakia	s.positive	Y	+	-	increased	vesicular	+	regular	N	benign	leukoplakia
62	1173/09	75	F	Y	N	Y	N	N	Y	Y	N	N	5	Y	N	Y	50	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
63	1187/09	60	F	Y	N	Y	N	Y	Y	Y	Y	N	3	N	Y	Y	25	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	Y	malignant	WDSCC
64	1199/09	62	F	N	Y	Y	N	N	N	N	N	N	1	N	N	Y	12	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
65	1212/09	48	F	Y	N	Y	Y	N	Y	Y	Y	Y	9	N	N	N	20	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	normal	vesicular	+	irregular	N	malignant	WDSCC
66	1221/09	48	M	Y	N	Y	N	N	N	Y	N	N	4	Y	Y	Y	16	hard palate	<2cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
67	1227/09	55	F	N	N	Y	Y	N	Y	Y	Y	N	7	N	N	Y	26	tongue lateral bo	<2cm	ulceroproliferative	erythroplaki	s.positive	Y	+	-	increased	hyperchromatic	-	irregular	N	malignant	erythroplakia
68	1231/09	40	F	Y	N	Y	N	N	N	Y	Y	N	8	N	N	Y	23	lower alveolus	<2cm	ulceroproliferative	ca	s.positive	Y	+	-	increased	vesicular	+	regular	N	s.malignar	verrucous ca
69	1232/09	60	F	Y	N	Y	Y	N	Y	Y	Y	N	5	N	Y	N	35	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	hyperchromatic	-	irregular	N	malignant	MDSCC
70	1253/09	75	M	Y	N	Y	N	N	N	Y	Y	N	4	N	Y	Y	40	buccal mucosa	<2cm	ulceroproliferative	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
71	1255/09	62	F	N	N	Y	N	N	Y	Y	Y	Y	12	N	Y	Y	38	buccal mucosa	2-4cm	ulceroproliferative	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
72	1307/09	60	F	Y	N	Y	Y	N	Y	Y	Y	Y	9	N	N	Y	37	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC

73	1347/09	70	F	Y	N	Y	N	N	Y	Y	Y	N	13	N	N	Y	40	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
74	1349/09	30	M	Y	Y	N	N	N	N	N	N	N	3	N	Y	Y	10	buccal mucosa	<2cm	plaque	erythroplaki	w.positive	Y	+	-	normal	normal	-	regular	N	benign	erythroplakia
75	1416/09	80	F	Y	N	Y	N	N	Y	Y	Y	N	8	N	Y	Y	50	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
76	1426/09	30	F	Y	N	Y	N	Y	Y	Y	Y	N	5	Y	N	N	12	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
77	1513/09	38	M	N	N	Y	N	N	Y	Y	Y	N	14	Y	Y	Y	14	tongue base	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
78	1541/09	50	F	Y	N	Y	N	N	Y	Y	N	N	3	N	N	Y	27	buccal mucosa	<2cm	ulceroproliferative	erythroplaki	s.positive	Y	+	-	normal	normal	+	irregular	N	benign	erythroplakia
79	1571/09	35	F	Y	N	Y	N	N	Y	Y	Y	Y	14	N	N	Y	10	floor of mouth	<2cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
80	1601/09	40	M	Y	N	Y	N	N	Y	Y	Y	N	3	Y	Y	N	20	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
81	1613/09	50	M	N	Y	N	N	N	N	N	N	N	4	Y	Y	Y	10	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	malignant	leukoplakia
82	1614/09	41	M	Y	N	Y	N	N	Y	Y	Y	Y	6	Y	Y	Y	20	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
83	1646/09	50	F	Y	N	Y	Y	N	Y	Y	Y	N	10	N	N	Y	15	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
84	1704/09	55	F	Y	N	Y	N	N	Y	Y	N	N	6	N	N	N	23	upper alveolus	<2cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
85	1705/09	56	M	Y	N	Y	Y	N	N	N	N	N	3	Y	Y	Y	25	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	MDSCC
86	1712/09	37	F	Y	N	Y	Y	Y	Y	Y	N	N	8	N	N	Y	15	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
87	1736/09	41	M	Y	N	Y	Y	N	Y	Y	Y	N	7	Y	Y	Y	22	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
88	1773/09	40	F	N	Y	N	N	N	N	N	N	N	4	N	N	Y	10	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
89	1810/09	65	F	Y	N	Y	N	N	Y	Y	Y	N	5	N	Y	Y	25	buccal mucosa	<2cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	normal	+	irregular	N	malignant	MDSCC
90	1833/09	78	F	N	N	Y	N	N	Y	Y	Y	Y	6	Y	N	Y	40	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	-	irregular	N	malignant	MDSCC
91	1854/09	50	F	Y	N	Y	Y	N	Y	Y	Y	N	5	N	N	Y	25	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
92	1860/09	87	F	Y	Y	N	N	N	N	N	N	N	2	N	N	Y	30	tongue ant2/3rd	<2cm	plaque	erythroplaki	w.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	erythroplakia
93	1902/09	60	F	Y	N	N	N	N	Y	N	N	Y	5	Y	N	Y	30	tongue ant2/3rd	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
94	1909/09	60	M	Y	Y	N	N	N	N	N	N	N	2	Y	Y	Y	24	tongue ant2/3rd	<2cm	ulcer	ca	s.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	chronic ulcer
95	1934/09	60	F	N	N	Y	N	N	Y	Y	Y	N	5	N	N	Y	30	upper alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	-	irregular	N	malignant	MDSCC
96	1957/09	55	F	Y	N	Y	Y	N	Y	Y	N	N	3	N	N	Y	20	tongue ant2/3rd	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
97	2033/09	53	M	Y	N	Y	N	N	Y	Y	Y	N	4	Y	Y	Y	24	floor of mouth	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
98	2162/09	64	M	Y	N	Y	N	N	Y	Y	Y	N	3	Y	Y	Y	30	tongue ant2/3rd	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
99	2201/09	50	F	Y	N	Y	Y	N	Y	Y	Y	Y	4	N	N	Y	30	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
100	2206/09	45	F	N	Y	N	N	N	N	N	N	N	5	N	N	Y	13	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
101	2214/09	55	F	Y	N	Y	N	N	Y	Y	Y	N	6	N	N	Y	24	tongue ant2/3rd	<2cm	ulcer	ca	s.positive	Y	+	+	normal	hyperchromatic	-	irregular	N	malignant	MDSCC
102	2251/09	63	M	Y	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	40	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
103	2281/09	45	F	Y	N	Y	N	N	Y	Y	Y	Y	3	N	Y	Y	25	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
104	2318/09	39	M	N	N	Y	N	N	N	N	N	N	4	N	N	N	0	tongue	<2cm	ulcer	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	mucoepidermoid ca
105	2522/09	70	F	Y	N	Y	N	N	Y	Y	Y	N	2	N	N	Y	34	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
106	2575/09	65	F	Y	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	32	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
107	2628/09	45	M	Y	N	Y	N	N	Y	N	Y	N	5	Y	Y	Y	20	floor of mouth	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
108	2651/09	35	F	Y	N	Y	N	N	Y	Y	Y	N	2	Y	Y	Y	12	RMT	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
109	53/10	65	F	N	N	Y	N	N	Y	N	Y	Y	5	N	N	Y	26	lower alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	MDSCC
110	95/10	55	F	Y	N	Y	N	N	Y	Y	Y	N	3	N	Y	Y	30	lower alveolus	<2cm	ulcer	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	WDSCC
111	115/10	60	M	N	Y	N	Y	N	N	N	N	N	6	N	Y	Y	24	buccal mucosa	2-4cm	plaque	submucosal	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
112	133/09	65	F	Y	Y	N	N	N	Y	Y	Y	N	3	N	Y	Y	33	hard palate	<2cm	plaque	ca	s.positive	Y	+	-	normal	normal	-	regular	N	benign	chronic ulcer

113	134/10	60	F	N	Y	N	N	N	N	N	N	N	2	N	N	Y	10	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
114	154/10	35	F	Y	N	Y	N	N	Y	Y	Y	Y	5	N	N	Y	22	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	MDSCC
115	156/10	30	F	Y	N	Y	N	N	N	N	N	N	3	N	N	N	0	hard palate	<2cm	ulcer	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	mucoepidermoid ca
116	194/10	60	F	Y	N	Y	N	N	Y	Y	N	N	7	N	N	Y	34	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
117	288/10	60	F	Y	N	Y	Y	N	Y	Y	N	N	5	N	Y	Y	26	hard palate	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
118	311/10	40	F	N	Y	N	N	N	N	N	N	N	1	N	N	Y	14	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
119	312/10	71	F	N	N	Y	N	N	Y	Y	Y	N	5	N	N	Y	40	hard palate	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
120	317/10	35	F	Y	N	Y	N	Y	Y	Y	Y	N	4	N	N	Y	10	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
121	334/10	55	F	Y	N	Y	N	N	Y	Y	Y	N	6	N	N	Y	21	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
122	404/10	45	F	Y	N	Y	N	N	Y	Y	Y	N	5	N	N	Y	18	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
123	410/10	55	F	Y	N	Y	N	N	N	Y	N	N	4	N	N	Y	22	buccal mucosa	<2cm	ulceroproliferative	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	carcinoma in situ
124	442/10	35	F	Y	N	Y	N	N	Y	Y	N	N	6	N	N	Y	11	RMT	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
125	454/1050	50	F	Y	N	Y	N	N	Y	Y	Y	N	5	N	N	Y	24	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
126	473/10	70	F	Y	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	40	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
127	474/10	48	F	N	N	Y	N	N	Y	Y	Y	N	4	N	N	Y	10	buccal mucosa	>4 cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
128	481/10	40	F	Y	N	Y	N	N	Y	Y	Y	N	6	N	N	Y	23	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	Y	malignant	WDSCC
129	482/10	35	F	Y	N	Y	N	N	Y	Y	N	N	4	N	N	Y	14	tongue ant2/3rd	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
130	484/10	48	F	Y	N	Y	N	N	Y	Y	Y	Y	5	N	N	Y	22	upper alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
131	485/10	50	F	Y	Y	N	N	N	N	N	N	N	3	N	N	Y	10	buccal mucosa	<2cm	plaque	erythroplaki	w.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	erythroplakia
132	503/10	75	F	Y	N	Y	N	N	Y	Y	Y	N	4	N	N	Y	38	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
133	510/10	35	F	Y	N	Y	N	N	Y	N	Y	N	7	N	N	Y	12	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
134	528/10	65	M	Y	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	24	buccal mucosa	<2cm	ulcer	leukoplakia	w.positive	Y	+	-	normal	hyperchromatic	-	regular	N	s.malignar	dysplasia
135	535/10	36	M	N	Y	N	N	N	N	N	Y	N	5	Y	N	Y	12	buccal mucosa	<2cm	plaque	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
136	544/10	53	M	Y	N	Y	N	N	Y	Y	N	N	6	N	Y	Y	26	floor of mouth	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
137	595/10	50	F	N	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	23	buccal mucosa	<2cm	plaque	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
138	612/10	55	F	N	Y	N	N	N	N	N	N	N	5	N	N	Y	16	buccal mucosa	<2cm	plaque	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
139	620/10	50	F	Y	N	Y	N	N	Y	Y	N	N	4	N	N	Y	23	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
140	625/10	65	M	N	Y	N	N	N	N	N	N	N	3	Y	N	Y	11	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
141	631/10	36	F	Y	N	Y	N	N	N	N	N	N	2	N	N	N	0	hard palate	<2cm	ulcer	ca	w.positive	Y	+	-	normal	normal	-	regular	N	benign	mucoepidermoid ca
142	640/10	60	F	N	Y	N	N	N	N	N	N	N	4	N	Y	Y	26	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
143	658/10	50	F	Y	N	Y	N	N	Y	Y	Y	Y	6	N	Y	Y	16	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	hyperchromatic	+	irregular	N	malignant	MDSCC
144	664/10	45	F	N	N	Y	N	N	N	N	Y	N	5	N	N	Y	14	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	verrucous ca
145	678/10	45	F	Y	N	Y	N	N	Y	N	Y	N	4	N	N	Y	22	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
146	680/10	45	F	Y	N	Y	N	N	N	N	Y	N	5	N	N	Y	21	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
147	696/10	65	M	Y	N	Y	N	N	Y	N	Y	N	5	Y	N	Y	35	RMT	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
148	703/10	80	F	Y	Y	N	N	N	N	N	N	N	4	N	N	Y	24	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
149	704/10	90	F	Y	Y	N	N	N	Y	N	Y	Y	4	N	N	Y	39	buccal mucosa	<2cm	plaque	erythroplaki	w.positive	Y	+	+	normal	normal	-	regular	N	s.malignar	erythroplakia
150	705/10	65	F	Y	N	Y	N	N	Y	Y	Y	Y	5	N	Y	Y	23	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
151	719/10	60	F	Y	N	Y	N	N	Y	Y	Y	N	3	N	N	Y	24	upper alveolus	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	MDSCC
152	768/10	80	F	N	Y	N	N	N	N	N	Y	N	4	N	Y	Y	24	buccal mucosa	<2cm	ulcer	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	s.malignar	dysplasia

153	769/10	60	F	N	N	Y	N	N	Y	N	Y	N	5	N	N	Y	34	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
154	771/10	65	F	Y	N	Y	N	N	Y	Y	Y	Y	7	N	Y	Y	22	RMT	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
155	774/10	45	F	Y	N	Y	N	N	N	Y	Y	Y	8	N	N	Y	14	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
156	787/10	70	F	Y	N	Y	N	Y	Y	Y	N	N	3	N	N	Y	45	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
157	789/10	60	F	Y	N	Y	N	N	Y	N	N	Y	4	N	N	Y	23	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
158	849/10	56	F	Y	N	Y	N	N	Y	Y	N	Y	5	N	N	Y	24	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
159	861/10	60	F	Y	N	Y	N	N	Y	N	N	N	8	N	N	Y	21	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
160	867/10	65	M	Y	N	Y	Y	N	N	Y	Y	N	3	Y	N	Y	32	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
161	996/10	60	M	Y	N	Y	N	N	Y	Y	Y	N	4	Y	Y	Y	23	tongue post 1/3	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
162	1005/10	55	F	Y	N	Y	N	N	Y	Y	N	N	6	N	N	Y	25	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	Y	malignant	WDSCC
163	1023/10	45	F	Y	N	Y	N	N	N	N	N	N	5	N	N	Y	23	buccal mucosa	<2cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
164	1064/10	52	F	Y	N	Y	N	N	N	N	N	N	8	N	N	Y	18	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
165	1072/10	70	M	Y	N	Y	N	N	N	N	N	N	6	Y	Y	Y	48	upper alveolus	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
166	1075/10	82	F	Y	N	Y	N	N	Y	Y	N	N	3	N	N	Y	53	buccal mucosa	2-4cm	ulceroproliferative	ca	s.positive	Y	-	+	increased	vesicular	+	irregular	N	malignant	WDSCC
167	1094/10	60	F	Y	N	Y	N	N	N	N	N	N	10	N	Y	Y	28	buccal mucosa	>4cm	ulceroproliferative	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	MDSCC
168	1118/10	58	M	N	Y	N	N	N	N	N	N	N	3	Y	N	Y	34	lower alveolus	<2cm	plaque	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
169	1135/10	45	F	Y	Y	N	N	N	N	N	N	N	5	N	N	Y	14	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	leukoplakia
170	1169/10	40	F	Y	N	Y	N	N	N	N	N	N	4	N	N	Y	15	buccal mucosa	<2cm	ulcer	leukoplakia	negative	Y	+	-	normal	normal	-	regular	N	s.malignar	dysplasia
171	1185/10	40	F	Y	N	Y	N	N	Y	Y	N	N	6	N	N	Y	18	buccal mucosa	2-4cm	ulcer	ca	s.positive	Y	+	+	increased	vesicular	+	irregular	N	malignant	WDSCC
172	1409/10	55	F	N	Y	N	N	N	N	N	N	N	4	N	N	Y	31	buccal mucosa	<2cm	plaque	leukoplakia	w.positive	Y	+	-	normal	normal	-	regular	N	benign	microinvasive ca